jc781 U.S. PTO
10/06/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING APPLICATION UNDER RULE 1.53(b)

Pursuant to 37 CFR 1.53(b), please file a ☒ continuation-in-part of the pending prior PATENT APPLICATION of:
Inventor: YOKOI et al
Serial No.08/765,337
Filed: October 6, 2000
For: **HG-CSF FUSION POLYPEPTIDE HAVING C-MPL ACTIVITY, DNA CODING FOR SAME AND METHODS OF TREATING ANEMIA USING SAME(AS AMENDED)**

Atty Dkt.: 249-118
C# M#
Date: October 6, 2000
Group: 1646
Examiner: Mertz

jc825 U.S. PTO
09/680514
10/06/00

Assistant Commissioner for Patents
Washington, DC 20231
Sir:

This request for filing under Rule 53(b) is made by the following named inventor(s) (using the above-identified title):
Inventor(s): YOKOI et al

☒ Attached is a true copy of the prior application as originally filed including the specification, claims, Oath/Declaration and drawings (if any) and abstract (if any). No amendments (if any) referenced in the Oath or Declaration filed to complete the prior application introduced new matter.

☒ Priority is hereby claimed under 35 USC 119 based on the following foreign applications, the entire content of which is hereby incorporated by reference in this application:

Application Number	Country	Day/Month/Year/Filed
Hei 7-102625	Japan	26 April 1995
PCT/JP96/01157		26 April 1996

☐ certified copy(ies) of foreign application(s) attached or
☐ already filed on _____ in prior appln. no. _____ filed _____
☒ already filed in PCT/JP96/01157 filed 26 April 1996

☒ The prior application is assigned to Kyowa Hakko Kogyo Co., Ltd..
☒ Power of Attorney has been granted to Arthur R. Crawford et al, Reg. No. 25,327 of Nixon & Vanderhye P.C., 1100 N. Glebe Rd., 8th Flr, Arlington, VA 22201.
☒ Address all future communications to: Nixon & Vanderhye P.C., 1100 N. Glebe Rd., 8th Floor, Arlington, VA 22201.
☒ Please amend the specification by inserting before the first line --This is a continuation-in-part of application Serial No. 09/765,337, filed December 23, 1996, now pending, the entire content of which is hereby incorporated by reference in this application.--
☐ "Small entity" statement of record. ☐ "Small entity" statement attached.
☐ Petition filed in prior application to extend its life to insure copendency.
☒ The Examiner's attention is directed to the prior art cited in the parent application by applicant and/or Examiner for the reasons stated therein.
☒ Please enter the attached and/or below preliminary amendment **prior** to calculation of filing fee:
Attached Preliminary Amendment, Information Disclosure Statement, Declaration re Deposited Materials, Letter re transfer of sequence listing from parent case and copy of sequence listing.

☒ The entire disclosure of the prior application above-referenced is considered as being part of the disclosure of this new application and is hereby incorporated by reference therein.

FILING FEE IS BASED ON CLAIMS AS FILED LESS ANY HEREWITH CANCELED

Basic Filing Fee				$	710.00
Total effective claims	9	- 20 (at least 20) =	0	x $ 18.00	$ 0.00
Independent claims	2	- 3 (at least 3) =	0	x $ 80.00	$ 0.00
If any proper multiple dependent claims now added for first time, add $270.00 (ignore improper)					$ 0.00
				SUBTOTAL	$ 710.00
If "small entity," then enter half (1/2) of subtotal and subtract					-$(0.00)
				SECOND SUBTOTAL	$ 710.00
Assignment Recording Fee ($40.00)					$ 0.00
				TOTAL FEE ENCLOSED	$ 710.00

Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140.** A duplicate copy of this sheet is attached.

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NIXON & VANDERHYE P.C.
By Atty: Arthur R. Crawford, Reg. No. 25,327

Signature: _____

464402

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re CIP of Patent Application Serial No. 08/765,337 of

YOKOI et al — Atty. Ref.: 249-118

Serial No. to be assigned — Group: 1646

Filed: October 6, 2000 — Examiner: Mertz

For: hG-CSF FUSION POLYPEPTIDE HAVING c-mpl ACTIVITY, DNA CODING FOR SAME AND METHODS OF TREATING ANEMIA USING SAME(As Amended)

* * * * * * * * * * * *

October 6, 2000

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

In order to place the above-identified application in better condition for examination, please amend the application as follows:

IN THE TITLE

Amend the title to read as follows: -- hG-CSF FUSION POLYPEPTIDE HAVING c-mpl ACTIVITY, DNA CODING FOR SAME AND METHODS OF TREATING ANEMIA USING SAME--

IN THE SPECIFICATION

Page 11, line 24, delete "No. 4, 5 or 6" and insert --NOS: 5, 7 or 9--.

Page 12, line 26, delete "No. 4, 5, or 6" and insert --NOS: 4, 6 or 8--.

Page 13, line 7, delete "No. 4, 5 or 6" and insert --NOS: 4, 6 or 8--; and

line 13, delete "No. 4, 5 or 6" and insert -- NOS: 4, 6 or 8--.

IN THE CLAIMS

Cancel claims 1-9 and add the following new claims:

--10. A fusion polypeptide which comprises a human granulocyte colony stimulating factor polypeptide and c-mpl ligand polypeptide encoded by a nucleotide sequence selected from the nucleotide sequence SEQ ID NOS: 4, 6 and 8 wherein the fusion polypeptide has no mouse IL-3 activity.

11. The fusion polypeptide according to claim 10 in which the human granulocyte colony stimulating factor polypeptide is fused via a spacer peptide to a c-mpl ligand polypeptide.

12. A fusion polypeptide encoded by a nucleotide sequence selected from the nucleotide sequences shown in SEQ ID NOS: 4, 6 and 8.

13. The fusion polypeptide of claim 10 chemically modified with a polyalkylene glycol derivative.

14. The fusion polypeptide according to claim 13 wherein the polyalkylene glycol derivative is a polyethylene glycol derivative, a polypropylene glycol derivative or a polyoxyethylene-polyoxypropylene copolymer derivative.

15. A pharmaceutical composition for treating anemia comprising the fusion polypeptide of claim 10 in a pharmaceutically acceptable carrier, vehicle or auxiliary agent.

16. A method of treating anemia comprising administering to a subject in need of same an effective amount of the fusion polypeptide of claim 10.

17. A method of simultaneously amplifying platelets and neutrophils comprising administering to a subject in need of same an effective amount of the fusion polypeptide of claim 10.

18. A method of controlling formation of megakaryocyte colonies and neutrophil colonies and/or controlling differentiation or maturation of megakaryocyte precursors and neutrophil precursors comprising administering to a subject in need of same an effective amount of the fusion polypeptide of claim 10.--.

REMARKS

This application is a continuation-in-part of application Serial No. 08/765,337 and is identical to the disclosure of that application with the exception of a single sentence added to page 5 of the specification, first full paragraph. This statement indicates that the fusion protein of the present invention has no mouse IL-3 activity. This is an inherent property of the fusion protein and is illustrated in the examples at pages 50 and 51. Applicants retain their priority rights to both the underlying PCT and Japanese priority applications in accordance with *In re Davies et al* 177 USPQ 381 (CCPA 1973).

The claims now before the examiner are the same claims that were presented in the parent application with the amendment filed July 27, 2000. The title of this application has been amended and also passages at pages 11-13 of the specification adjusted.

With this filing there is a comprehensive Information Disclosure Statement bringing forward the art of record in the parent application.

It is submitted that claims 10-18 are in condition for allowance. Favorable action is solicited.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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SPECIFICATION

NOVEL POLYPEPTIDES

TECHNICAL FIELD

The present invention relates to a fusion polypeptide comprising a polypeptide having a granulocyte colony stimulating factor (hereinafter referred to as "G-CSF") activity and a polypeptide having a platelet growth factor (thrombopoietin, hereinafter referred to as "TPO") activity, and DNA which codes for the fusion polypeptide. Since the fusion polypeptide of the present invention can form and amplify platelets and neutrophils simultaneously, it is useful for the treatment of anemia and the like.

BACKGROUND ART

Blood comprises hematopoietic cells such as erythrocytes, leukocytes, platelets and the like. These hematopoietic cells mature from only one kind of pluripotential blood stem cell through various differentiation steps. These steps undergo complex regulation by a group of proteinous factors which are generally referred to as cytokines. A certain type of cytokine takes part in the differentiation and multiplication of various hematopoietic cells. On the other hand, a certain type of hematopoietic cell undergoes regulation of its differentiation and multiplication by various types of cytokines. This is called overlapping cytokine actions. Among

these cytokine members, TPO and G-CSF are considered to have small overlapping actions.

TPO mainly takes part in the formation of platelets. Platelets are formed by the fragmentation of megakaryocytes, a hematopoietic cell which has a large nucleus and is present mainly in bone marrow. Platelets are essential for forming blood clots at damaged portions in blood vessels. Platelets also play important roles in not only blood coagulation but also injury healing by releasing proteins having other functions at the damaged portions. A significant decrease in the number of platelets may be fatal, because the body may easily bleed.

G-CSF is a cytokine which accelerates activation of neutrophils, a member of the leukocytes, and differentiation of neutrophils from their precursor cells. Neutrophils exert the first defense action when invaded by foreign enemies such as bacteria, viruses and the like. When the number of neutrophils is decreased, the body becomes defenseless against infection, and this too is also often fatal.

Current medical treatment of cancers often cause side effects in which pluripotential blood stem cells are damaged by the administration of a chemotherapeutic drug, irradiation of X-rays or bone marrow transplantation for the treatment of leukemia, thus decreasing the number of all hematopoietic cells. Apparently, it is markedly beneficial for thrombopenia and leukopenia patients to amplify the number of these cells by

the administration of cytokine, to suppress bleeding tendency and preventing infectious diseases.

A cytokine which can amplify platelets and neutrophils simultaneously has not been found, and there is no medicine having such an effect.

Leukemia inhibiting factors, stem cell factors, macrophage colony stimulating factors, granulocyte/macrophage colony stimulating factors, erythropoietin, interleukin (IL)-3, IL-6, IL-11, megakaryocyte colony stimulating factors and the like are known as substances which amplify platelets or enhance differentiation and multiplication of megakaryocytes [Metcalf *et al.*, *Blood*, 80, 50-56 (1990); Hunt *et al.*, *Blood*, 80, 904-911 (1992); Examined Japanese Patent Publication No. 6-11705; Hoffman *et al.*, *Blood Cells*, 13, 75-86 (1987); Mazur *et al.*, *Exp. Hematol.*, 15, 1123-1133 (1987); McNiece *et al.*, *Exp. Hematol.*, 16, 807-810 (1988); Lu *et al.*, *Brit. J. Hematol.*, 70, 149-156 (1988); Ishibashi *et al.*, *Proc. Natl. Acad. Sci. USA*, 86, 5953-5957 (1989); WO 95/21919; WO 95/18858]. It is understood that these many cytokine members amplify platelets by overlapping actions. Recently, it was revealed that a receptor ligand called c-mpl is a cytokine which has the highest activity among platelet amplifying factors and acts directly [de Sauvage *et al.*, *Nature*, 369, 533 (1994)].

As substances which multiply granulocytes, the above-mentioned IL-3, macrophage colony stimulating factors, granulocyte/macrophage colony stimulating factors and the like

are known, but G-CSF has the highest activity in terms of multiplying neutrophils selectively [Nicola *et al.*, *J. Biol. Chem.*, 258, 9017 (1983)]. With regard to a polypeptide in which two different kinds of cytokine are fused, there are reports in Japanese Published Unexamined International Patent Application No. 500116/94, U.S. Patent 5,359,035, *Exp. Hematol.*, 21, 647-655 (1993) and *ibid.*, 18, 615 (1990) and the like.

However, nothing is known about a fusion polypeptide in which TPO is used as one of the fused cytokines.

An object of the present invention is to provide a fusion polypeptide which can produce and amplify platelets and neutrophils simultaneously. This fusion polypeptide allows the formation of megakaryocyte colonies and neutrophil colonies and the differentiation or maturation of megakaryocyte precursor and neutrophil precursor can be controlled.

DISCLOSURE OF THE INVENTION

The present invention relates to a fusion polypeptide which comprises a polypeptide having G-CSF activity and a polypeptide having TPO activity and DNA which codes for the fusion polypeptide. Also disclosed are fusion polypeptides in which a polypeptide having G-CSF activity and a polypeptide having TPO activity are fused via a spacer peptide and DNA which codes for the fusion polypeptide; and a polypeptide in which the fusion polypeptide comprising a polypeptide having G-

CSF activity and a polypeptide having TPO activity is chemically modified with a polyethylene glycol derivative. Also provided are anemia-treating compositions containing the fusion polypeptide as an active ingredient.

The fusion protein of the present invention has no mouse IL-3 activity.

As the polypeptide having G-CSF activity for use in the present invention, any protein may be used with the proviso that it has the requisite G-CSF activity, such as a polypeptide having the amino acid sequence shown in Table 1 [*Nature,* 319, 415 (1986)] .

Also useful is a protein which has an amino acid sequence derived from the amino acid sequence shown in Table 1 by substitution, deletion or addition of one or more amino acids, and examples thereof include hG-CSF derivatives shown in Table 2 and described in Japanese published Unexamined Patent Application No. 267299/88, Japanese Published Unexamined Patent application No. 299/88, and Japanese Published Unexamined International Patent Application No. 500636/88.

TABLE 1

```
 X ThrProLeuGlyProAlaSerSerLeuProGlnSerPheLeuLeu
   1           5              10              15
LysCysLeuGluGlnValArgLysIleGlnGlyAspGlyAlaAlaLeu
            20              25              30
GlnGluLysLeuCysAlaThrTyrLysLeuCysHisProGluGluLeu
        35              40              45
ValLeuLeuGlyHisSerLeuGlyIleProTrpAlaProLeuSerSer
    50              55              60
CysProSerGlnAlaLeuGlnLeuAlaGlyCysLeuSerGlnLeuHis
65              70              75
SerGlyLeuPheLeuTyrGlnGlyLeuLeuGlnAlaLeuGluGlyIle
80              85              90              95
SerProGluLeuGlyProThrLeuAspThrLeuGlnLeuAspValAla
            100             105             110
AspPheAlaThrThrIleTrpGlnGlnMetGluGluLeuGlyMetAla
        115             120             125
ProAlaLeuGlnProThrGlnGlyAlaMetProAlaPheAlaSerAla
    130             135             140
PheGlnArgArgAlaGlyGlyValLeuValAlaSerHisLeuGlnSer
145             150             155
PheLeuGluValSerTyrArgValLeuArgHisLeuAlaGlnPro
160             165             170         174
```

(X represents H or Met.)

TABLE 2

Position from N-terminal amino acid (hG-CSF in Table 1)	Substituted amino acid in hG-CSF derivatives											
	a)	b)	c)	d)	e)	f)	g)	h)	i)	j)	k)	l)
1st (Thr)	*	Val	Cys	Tyr	Arg	*	Asn	Ile	Ser	*	Ala	*
3rd (Leu)	Glu	Ile	Ile	Ile	Thr	Thr	Glu	Thr	Thr	*	Thr	*
4th (Gly)	Lys	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Tyr	*
5th (Pro)	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	*	Arg	*
17th (Cys)	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser

*: unsubstituted amino acid

As the polypeptide having TPO activity for use in the present invention, any protein may be used with the proviso that it has the requisite TPO activity, such as the c-mpl ligand which is a polypeptide having the amino acid sequence shown in Table 3 [*Nature*, 369, 533 (1994)], as well as leukemia inhibiting factors, stem cell factors, macrophage colony stimulating factors, granulocyte/macrophage colony stimulating factors, erythropoietin, interleukin (IL)-3, IL-6, IL-11, megakaryocyte colony stimulating factors and the like.

TABLE 3

```
SerProAlaProProAlaCysAspLeuArgValLeuSerLysLeu
  1               5                  10                  15
LeuArgAspSerHisValLeuHisSerArgLeuSerGlnCysPro
             20                  25                  30
GluValHisProLeuProThrProValLeuLeuProAlaValAsp
             35                  40                  45
PheSerLeuGlyGluTrpLysThrGlnMetGluGluThrLysAla
             50                  55                  60
GlnAspIleLeuGlyAlaValThrLeuLeuLeuGluGlyValMet
             65                  70                  75
AlaAlaArgGlyGlnLeuGlyProThrCysLeuSerSerLeuLeu
             80                  85                  90
GlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGln
             95                 100                 105
SerLeuLeuGlyThrGlnLeuProProGlnGlyArgThrThrAla
            110                 115                 120
HisLysAspProAsnAlaIlePheLeuSerPheGlnHisLeuLeu
            125                 130                 135
ArgGlyLysValArgPheLeuMetLeuValGlyGlySerThrLeu
            140                 145                 150
CysValArgArgAlaProProThrThrAlaValProSerArgThr
            155                 160                 165
SerLeuValLeuThrLeuAsnGluLeuProAsnArgThrSerGly
            170                 175                 180
LeuLeuGluThrAsnPheThrAlaSerAlaArgThrThrGlySer
            185                 190                 195
GlyLeuLeuLysTrpGlnGlnGlyPheArgAlaLysIleProGly
            200                 205                 210
LeuLeuAsnGlnThrSerArgSerLeuAspGlnIleProGlyTyr
            215                 220                 225
LeuAsnArgIleHisGluLeuLeuAsnGlyThrArgGlyLeuPhe
            230                 235                 240
ProGlyProSerArgArgThrLeuGlyAlaProAspIleSerSer
            245                 250                 255
GlyThrSerAspThrGlySerLeuProProAsnLeuGlnProGly
            260                 265                 270
TyrSerProSerProThrHisProProThrGlyGlnTyrThrLeu
            275                 280                 285
ProLeuProProThrLeuProThrProValValGlnLeuHis
            290                 295                 300
ProLeuLeuProAspProSerAlaProThrProThrProThrSer
            305                 310                 315
ProLeuLeuAsnThrSerTyrThrHisSerGlnAsnLeuSerGln
            320                 325                 330
GluGly
  332
```

The polypeptide having G-CSF activity and the other polypeptide having TPO activity, which constitute the fused polypeptide of the present invention, are not particularly limited, provided that they contain respective activity-producing portions. For example, when the c-mpl ligand is used as the polypeptide having TPO activity, it may contain an amino acid sequence of the 153rd and 154th positions counting from the N-terminal amino acid.

Also included in the polypeptide of the present invention is a polypeptide in which a polypeptide having G-CSF activity and a polypeptide having TPO activity are fused via a spacer peptide. As the spacer peptide, any sequence may be used with the proviso that it does not spoil the G-CSF activity and TPO activity. For example, the peptide shown in Table 4 can be used as the spacer peptide.

TABLE 4

Linker
$(GlyGlyGlySer)_3Arg$
$(SerGlyGlyGly)_4Arg$
SerGlyGlyGlyArg
$(SerGlyGlyGly)_4$
SerGlyGlyGly
$(GlyGlyGlySer)_3$
$(GlyGlyGlySer)_2$

Examples of the fusion polypeptide of the present invention include a polypeptide having the amino acid sequence shown in Sequence ID No. 1, 2 or 3 and a polypeptide derived from the amino acid sequence of the fusion polypeptide by addition, deletion or substitution of one or more amino acids within such a range that the G-CSF activity and TPO activity are not spoiled, having a homology of 40% or more with the amino acid sequence of the polypeptide. The homology is preferably 60% or more, and more preferably 80% or more.

The substitution, deletion or addition of amino acids can be carried out in accordance with known methods described for example in *Nucleic Acid Research*, 10, 6487 (1982); *Proc. Natl. Acad. Sci., USA*, 79, 6409 (1982); *Proc. Natl. Acad. Sci., USA*, 81, 5662 (1984); *Science*, 224, 1431 (1984); PCT WO 85/00817; *Nature*, 316, 601 (1985); *Gene*, 34, 315 (1985); *Nucleic Acid Research*, 13, 4431 (1985); and "Current Protocols in Molecular Biology", Chap. 8, Mutagenesis of Cloned DNA, John Wiley & Sons, Inc. (1989).

Also included in the fusion polypeptide of the present invention is a peptide having an amino acid sequence in which a secretion signal peptide is added to the N-terminal amino acid of the above-mentioned polypeptide; examples include a polypeptide having the amino acid sequence shown in Sequence ID No. 4, 5 or 6.

In addition, a fusion polypeptide having G-CSF activity and TPO activity, in which at least one amino group of the

above-mentioned polypeptide is chemically modified with a polyalkylene glycol derivative, is also included in the fusion polypeptide of the present invention.

Examples of the polyalkylene derivative include a polyethylene glycol derivative, a polypropylene glycol derivative, a polyoxyethylene-polyoxypropylene copolymer derivative and the like. Polyethylene glycol-succinimidyl propionate is preferred.

The fusion polypeptide chemically modified with a polyethylene glycol derivative can be prepared in accordance with the method described in Japanese Examined Patent Publication No. 96558/95.

The DNA which codes for the fusion polypeptide (hereinafter referred to as "TPO-CSF") of the present invention can be obtained by polymerase chain reaction (PCR) and the like based on the known nucleotide sequences of a polypeptide having TPO activity and a polypeptide having G-CSF activity. It can also be obtained by chemical synthesis.

Examples of DNA which codes for TPO-CSF include a DNA containing a nucleotide sequence that codes for a polypeptide having the amino acid sequence shown in Sequence ID No. 1, 2 or 3 or a polypeptide derived from the amino acid sequence of the polypeptide by substitution, deletion or addition of one or more amino acids but having the G-CSF activity and TPO activity, such as a DNA which contains the nucleotide sequence shown in Sequence ID Nos. 4, 5 or 6.

Other examples are DNA's in which mutation such as substitution mutation, deletion mutation, insertion mutation or the like is introduced into the above-mentioned DNA within such a range that the G-CSF activity and TPO activity are not spoiled, which can be obtained, for example, by colony hybridization or plaque hybridization using a DNA containing the nucleotide sequence shown in Sequence ID No. 4, 5 or 6 as a probe.

An example is a DNA which is identified by carrying out hybridization of a membrane filter on which colony- or plaque-originated DNA is fixed, at 65°C in the presence of 0.7 to 1.0 M sodium chloride using a DNA containing the nucleotide sequence shown in Sequence ID No. 4, 5 or 6 as a probe, and subsequently washing the resulting filter at 65°C in 0.1 to 2-fold SSC solution (1-fold SSC contains 150 mM sodium chloride and 15 mM sodium citrate).

The hybridization techniques are described in "Molecular Cloning, A laboratory manual", second edition (edited by Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory Press, 1989).

All polypeptides encoded by the DNA defined in the foregoing are included in the TPO-CSF.

Examples of plasmids containing the TPO-CSF-encoding DNA include pBS-T153LND28, pBS-T154ND28 and pBS-T153ND28LN1. *Escherichia coli* TLN-1 as a colon bacillus containing pBS-T153LND28 and *Escherichia coli* TN-1 as a colon bacillus

containing pBS-T154ND28 have been deposited on February 16, 1995, in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan (the postal code: 305), and have been assigned the designations as FERM BP-5001 and FERM BP-5002, respectively.

In order to express the thus obtained TPO-CSF-encoding gene (hereinafter referred to as "TPO-CSF gene") in a host, a DNA fragment containing the TPO-CSF gene is first cleaved into a TPO-CSF gene-containing DNA of an appropriate length with restriction enzymes or DNA hydrolyzing enzymes and inserted into downstream site of a promoter gene on an expression vector and then the thus DNA-inserted expression vector is introduced into a host suitable for the expression vector.

As the host, any host capable of expressing the intended gene can be used. Examples thereof include microbial strains belonging to the genera *Escherichia*, *Serratia*, *Corynebacterium*, *Brevibacterium*, *Pseudomonas*, *Bacillus* and the like, as well as yeast strains, animal cell hosts and the like.

Useful as the expression vector is a vector which can replicate by itself in the above-mentioned host or can be inserted into its chromosome and has a promoter at a site where transcription of the TPO-CSF gene can be made.

When a microorganism such as *Escherichia coli* or the like is used as the host, it is desirable that the TPO-CSF expression vector can replicate by itself in the microorganism

and comprises a promoter, a ribosome binding sequence, the TPO-CSF gene and a transcription termination sequence. It may also contain a regulatory gene.

Examples of the expression vector include pBTrp2, pBTac1 and pBTac2 (all available from Boehringer-Mannheim Co.), pKYP10 (Japanese Published Unexamined Patent Application No. 110600/83), pKYP200 [*Agric. Biol. Chem.*, 48, 669 (1984)], pLSA1 [*Agric. Biol. Chem.*, 53, 277 (1989)], pGEL1 [*Proc. Natl. Acad. Sci., USA*, 82, 4306 (1985)], pBluescript (available from STRATAGENE Co.), pTrs30 [prepared from *Escherichia coli* JM109/pTrs30 (FERM BP-5407)], pTrs32 [prepared from *Escherichia coli* JM109/pTrs32 (FERM BP-5408)], pAGE107 [Japanese Published Unexamined Patent Application No. 22979/91; Miyaji *et al.*, *Cytotechnology*, 3, 133 (1990)], pAS3-3 (Japanese Published Unexamined Patent Application No. 227075/90) and pAMoERC3Sc CDM8 [Brian Seed *et al.*, *Nature*, 329, 840 (1987)].

As the promoter, any one capable of exerting expression in a host such as *Escherichia coli* or the like can be used. Examples thereof include promoters originated from *Escherichia coli*, phages and the like, such as trp promoter (Ptrp), lac promoter (Plac), P_L promoter, P_R promoter and the like. Also useful are artificially designed and modified promoters such as a promoter prepared by connecting two Ptrp promoters in series (Ptrpx 2), tac promoter and the like.

As the ribosome binding sequence, any sequence capable of exerting expression in a host such as *Escherichia coli* or

the like can be used, but it is desirable to use a plasmid in which the ribosome binding sequence and the initiation codon are arranged with an appropriate distance (for example, 6 to 18 bases).

Any gene which codes for TPO-CSF can be used as the TPO-CSF gene, but it is desirable to use the gene by substituting its bases in such a manner that the DNA sequence of the gene has codons most suitable for its expression in host microorganisms.

Although the transcription termination sequence is not always necessary for the expression of the gene, it is desirable to arrange the transcription termination sequence preferably just downstream of the structural gene.

Examples of the host include *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* DH5 α, *Escherichia coli* MC1000, *Escherichia coli* KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No. 49, *Escherichia coli* W3110, *Escherichia coli* NY49, *Bacillus subtilis*, *Bacillus amyloliquefacience*, *Brevibacterium immariophilum* ATCC 14068, *Brevibacterium saccharolyticum* ATCC 14066, *Brevibacterium flavum* ATCC 14067, *Brevibacterium lactofermentum* ATCC 13869, *Corynebacterium glutamicum* ATCC 13032, *Corynebacterium acetoacidophilum* ATCC 13870, *Microbacterium ammoniaphilum* ATCC 15354 and the like.

When a yeast strain is used as the host, YEp13 (ATCC 37115), YEp24 (ATCC 37051), YCp50 (ATCC 37419) or the like may be used as the expression vector.

Any type of promoter can be used, provided that it can exert expression in yeast strain hosts. Examples thereof include promoters of genes of hexose kinase and the like glycolytic pathway enzymes, gal 1 promoter, gal 10 promoter, heat shock protein promoter, MF α1 promoter, CUP 1 promoter and the like.

Examples of the host include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans*, *Schwanniomyces alluvius* and the like.

When animal cells are used as the host, examples of useful expression vectors include pcDNA I/Amp, pcDNA I, pcDM8 (all available from Funakoshi Co., Ltd.), pcDNA 3 (available from Invitrogen Co.), pAGE248, pAGE210 and the like.

Any promoter capable of exerting expression in the animal cell hosts can be used. For example, the promoter of human CMV IE (immediate early) gene may be used. Also, the enhancer of human CMV IE gene may be used together with the promoter.

Any gene which codes for TPO-CSF can be used as the TPO-CSF gene.

In general, only a portion of TPO-CSF expressed from the gene is secreted into the extracellular moiety, so that, in order to effect positive extracellular secretion of TPO-CSF

from the host, it is desirable to prepare and use a gene having a sequence in which a nucleotide sequence coding for a signal peptide is added to the gene, in accordance with the method of Paulson *et al.* [C. Paulson *et al.*, *J. Biol. Chem.*, 264, 17619 (1989)] and the method of Lowe *et al.* [John. B. Lowe *et al.*, *Proc. Natl. Acad. Sci., USA*, 86, 8227 (1989); John. B. Lowe *et al.*, *Genes Develop.*, 4, 1288 (1990)].

As the host, namalwa cells, HBT5637 (Japanese Published Unexamined Patent Application No. 299/88), COS cells, CHO cells and the like may be used.

Introduction of TPO-CSF gene-containing DNA into animal cells can be effected by any method, provided that it can introduce DNA into animal cells. For example, an electroporation method [Miyaji *et al.*, *Cytotechnology*, 3, 133 (1990)], a calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90), a lipofection method [Philip L. Felgner *et al.*, *Proc. Natl. Acad. Sci., USA*, 84, 7413 (1987)] and the like may be used. Isolation and cultivation of a transformant can be effected in accordance with the method described in Japanese Published Unexamined Patent Application No. 227075/90 or Japanese Published Unexamined Patent Application No. 257891/90.

TPO-CSF can be produced by cultivating the thus obtained transformant in accordance with the usually used cultivating method.

When a transformant obtained by using *Escherichia coli*, yeast or the like microorganism as the host is cultivated, the medium may be either a natural medium or a synthetic medium, with the proviso that it contains carbon sources, nitrogen sources, inorganic salts and the like which can be assimilated by the microorganism and cultivating of the transformant can be made efficiently.

As the carbon sources, those which can be assimilated by respective microorganisms are used, which include carbohydrates such as glucose, fructose, sucrose, molasses containing them, starch, starch hydrolyzates and the like, organic acids such as acetic acid, propionic acid and the like and alcohols such as ethanol, propanol and the like.

Examples of useful nitrogen sources include ammonia, ammonium salts of various inorganic and organic acids, such as ammonium chloride, ammonium sulfate, ammonium acetate, ammonium phosphate and the like, and other nitrogen-containing compounds, as well as peptone, meat extract, yeast extract, corn steep liquor, casein hydrolyzate, soybean cake and soybean cake hydrolyzate, various fermented microbial cells and digests thereof.

Examples of useful inorganic materials include potassium dihydrogenphosphate, dipotassium hydrogenphosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, calcium carbonate and the like.

Cultivation is carried out under aerobic conditions by shaking, submerged-aerial stirring or the like. The temperature for the cultivation is preferably 15 to 40°C, and the period for the cultivation is generally 16 to 96 hours. The medium pH is controlled at 3.0 to 9.0 during the cultivation. Adjustment of the pH is carried out using an inorganic or organic acid, an alkaline solution, urea, calcium carbonate, ammonia and the like.

As occasion demands, antibiotics such as ampicillin, tetracycline and the like may be added to the medium during the cultivation.

When a microorganism transformed with an expression vector prepared using an inducible promoter is cultivated, an inducer may be added to the medium as occasion demands. For example, isopropyl-β-D-thiogalactopyranoside (IPTG) or the like may be added to the medium when a microorganism transformed with an expression vector prepared using lac promoter is cultivated, or indoleacetic acid (IAA) or the like when a microorganism transformed with an expression vector prepared using trp promoter is cultivated.

When a transformant obtained using animal cells as the host is cultivated, generally used RPMI 1640 medium, MEM medium (manufactured by Eagle Co. or GibcoBRL Co.), D-MEM medium (manufactured by GibcoBRL Co.) or any one of these media further supplemented with fetal bovine serum and the like may be used.

The cultivation is carried out, for example, in the presence of 5% CO_2. The temperature for the cultivation is preferably 35 to 37°C, and the period for the cultivation is generally 3 to 7 days.

As occasion demands, antibiotics such as kanamycin, penicillin and the like may be added to the medium during the cultivation.

Productivity can be increased using a gene amplification system in which dihydrofolate reductase gene and the like are used, in accordance with the method described in Japanese Published Unexamined Patent Application No. 227075/90.

The TPO-CSF of the present invention obtained in this manner can be purified by commonly used protein purification techniques.

For example, when the TPO-CSF is not secreted into outside moiety of the host cells, a culture broth of the transformant is subjected to centrifugation to collect cells in the culture broth, and the thus collected cells are washed and then disrupted using a sonicator, French press, Manton Gaulin homogenizer, Dynomil or the like, thereby obtaining a cell-free extract. Thereafter, the cell-free extract is subjected to centrifugation, and the TPO-CSF is purified from the resulting supernatant fluid making use of various techniques including salting out with ammonium sulfate or the like salt, anion exchange chromatography on diethylaminoethyl (DEAE)-Sepharose or the like, hydrophobic chromatography on Butylsepharose,

Phenylsepharose or the like, molecular sieve-aided gel filtration and various types of electrophoresis such as isoelectric focusing and the like.

When the TPO-CSF is secreted, purified TPO-CSF can be obtained from a culture filtrate of the transformant in the same manner as the case of the above-mentioned treatment of cell-free extract supernatant.

When produced in *Escherichia coli* cells, it can be purified efficiently by the combination of the above-mentioned method with the method described in Japanese Published Unexamined Patent Application No. 267292/88.

Also, it is possible to produce the TPO-CSF of the present invention in the form of its fusion protein with another protein and to purify the product by affinity chromatography using a substance having affinity for the fused protein. For example, it is possible to produce the TPO-CSF of the present invention as its fusion protein with protein A and purify it by an immunoglobulin G-aided affinity chromatography, in accordance with the method of Lowe *et al.* [John. B. Lowe *et al.*, *Proc. Natl. Acad. Sci., USA*, 86, 8227 (1989); John. B. Lowe *et al.*, *Genes Develop.*, 4, 1288 (1990)].

In addition, it can also be purified by affinity chromatography using antibodies specific for a polypeptide which has G-CSF activity, such as antibodies specific for G-CSF.

The TPO-CSF of the present invention can be used as it is or as pharmaceutical compositions in various dosage forms.

The pharmaceutical compositions of the present invention are produced by mixing an effective amount of TPO-CSF as the active ingredient uniformly with pharmacologically acceptable carriers.

Preferably, these pharmaceutical compositions may be prepared in the form of unit dose packages suitable for injection.

Injections for use in injection administration can be prepared by using a carrier such as distilled water, a salt solution of sodium chloride or of a mixture of sodium chloride with other inorganic salts, a sugar solution of mannitol, lactose, dextran, glucose or the like, an amino acid solution of glycine, arginine or the like, an organic acid solution, an organic base solution or a mixture solution comprising a salt solution and a sugar solution. In that case, the composition can be made into solutions, suspensions or dispersions in the usual way using auxiliaries which include an osmotic pressure adjusting agent, a plant oil such as sesame oil or soybean oil and a surface active agent such as lecithin or a nonionic surface active agent. These solutions can be made into solid preparations by powder making, freeze drying and the like means, which are dissolved again prior to their use.

The above-mentioned pharmaceutical compositions which contain the TPO-CSF of the present invention as the active

ingredient are useful for the treatment anemia or patients who become anemic as a result of treatment of diseases.

BRIEF EXPLANATION OF THE DRAWINGS

Fig. 1 is an illustration showing construction of a plasmid containing DNA which codes for TPO-ND28 (1).

Fig. 2 is an illustration showing construction of a plasmid containing DNA which codes for TPO-ND28 (2).

Fig. 3 is an illustration showing construction of a plasmid containing DNA which codes for TPO-ND28 (3).

BEST MODE OF CARRYING OUT THE INVENTION

Example 1 Preparation of DNA which codes for TPO-CSF

A DNA which codes for TPO-CSF was prepared in the following manner, using a DNA which codes for a polypeptide ND28 in which the 1st position amino acid residue of the amino acid sequence of human G-CSF was substituted by alanine (Ala), and the 3rd position amino acid by threonine (Thr), the 4th position amino acid by tyrosine (Tyr), the 5th position amino acid by arginine (Arg) and the 17th position amino acid by serine (Ser) (Japanese Published Unexamined Patent Application No. 267292/88) as a DNA which codes for a polypeptide having G-CSF activity, and a DNA that codes for a polypeptide having the amino acid sequence of Table 3 (de Sauvage *et al.*, *Nature*, 369, 533 (1994); hereinafter referred to as "TPO") as a DNA which codes for a polypeptide having TPO activity. The fusion

polypeptide of TPO and ND28 is abbreviated as TPO-ND28 hereinafter.

1. Preparation of TPO gene

A TPO-encoding gene (hereinafter referred to as "TPO gene") for use in the preparation of TPO-ND28 was obtained by PCR in the following manner on the basis of the nucleotide sequence reported by de Sauvage *et al.* [*Nature*, 369, 533 (1994)].

A DNA shown in Sequence ID No. 7 containing 5' end nucleotide sequence of the TPO gene (hereinafter referred to as "primer 1") and a DNA shown in Sequence ID No. 8 containing 3' end nucleotide sequence of the TPO gene (hereinafter referred to as "primer 2") were synthesized using 380A DNA synthesizer of Applied Biosystems, Inc. In order to facilitate the cloning, a restriction enzyme recognition sequence was added to the terminus of each primer.

Amplification and cloning of the TPO gene translation region sequence were carried out by reverse transcription PCR using the primers 1 and 2, human liver poly A^+ mRNA (manufactured by Clontech Co., product No. CL 6510-1) mRNA and SuperScript Preamplification System for First Strand cDNA Synthesis Kit (manufactured by GibcoBRL Co.).

A 0.013 ml portion of aqueous solution containing 1,000 ng of human liver poly A^+ mRNA and 500 ng of oligo(dt) 12-18 (included in the kit) was treated at 70°C for 10 minutes and then allowed to stand in ice for 1 minute.

The resulting solution was mixed with 0.002 ml of ten times-concentrated synthesis buffer, 0.001 ml of 10 mM dNTP mix, 0.002 ml of 0.1 M DTT and 0.001 ml of SuperScript II RT (200 kU/ml) (all included in the kit), and the mixture was allowed to stand at room temperature for 10 minutes and then incubated at 42°C for 50 minutes. After completion of the incubation, the mixture was heated at 90°C for 5 minutes to terminate the reverse transcription reaction.

The reaction solution was mixed with 0.001 ml of E. coli RNase H (2,000 U/ml; included in the kit) and incubated at 37°C for 20 minutes.

A 0.1 ml portion of a reaction solution containing 0.005 ml of the above reaction solution, 400 nM of the primer 1, 400 nM of the primer 2, 20 mM of Tris-HCl (pH 8.2), 10 mM of potassium chloride, 0.01 mg/ml of bovine serum albumin (hereinafter referred to as "BSA"), 2 mM of magnesium chloride, 6 mM of ammonium sulfate, 0.1% Triton X-100, 10% dimethyl sulfoxide (hereinafter referred to as "DMSO"), 0.05 mM of deoxyadenosine triphosphate (hereinafter referred to as "dATP"), 0.05 mM of deoxycytidine triphosphate (hereinafter referred to as "dCTP"), 0.05 mM of deoxyguanosine triphosphate (hereinafter referred to as "dGTP") and 0.05 mM of deoxythymidine triphosphate (hereinafter referred to as "dTTP") was mixed with 2.5 units of Pfu polymerase (manufactured by Stratagene Co.) to carry out PCR using PERKIN ELMER CETUS DNA Thermal Cycler (manufactured by Takara Shuzo Co., Ltd.) by 35

time repetition of a three step incubation at 94°C for 45 seconds, at 50°C for 1 minute and at 72°C for 2 minutes.

The resulting reaction solution was subjected to phenol/chloroform extraction and ethanol precipitation, and the thus obtained precipitate was dissolved in 0.015 ml of TE buffer [10 mM Tris-HCl (pH 8.0) and 1 mM ethylenediaminetetraacetic acid (hereinafter referred to as "EDTA")].

The thus prepared solution was mixed with restriction enzymes HindIII and KpnI to cleave the DNA amplified by PCR.

The resulting solution was subjected to an agarose gel electrophoresis, and a HindIII-KpnI treated DNA of about 1.1 kb was isolated from the agarose gel.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the thus isolated DNA (50 ng) was ligated with a HindIII-KpnI cleaved 2.9 kb fragment (30 ng) of a plasmid vector pBlueScript II SK(-) having a multicloning site (manufactured by Stratagene Co.) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, an *Escherichia coli* strain DH5α (Library Efficiency DH5α Competent Cell, manufactured by GibcoBRL Co.) was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method [Birnboim *et al.*, *Nucleic Acids Res.*, 7, 1513 (1979)].

Nucleotide sequence of the insertion fragment in each plasmid was determined using Taq DyeDeoxy Terminator Cycle Sequencing Kit (manufactured by Applied Biosystems Japan Inc., product No. 401113) and ABI373A DNA Sequencer (manufactured by Applied Biosystems Japan Inc.). In determining the nucleotide sequence, six DNA's having the nucleotide sequences of Sequence ID Nos. 9 to 13 or 14 and two primers having the nucleotide sequence shown in Sequence ID No. 15 or 16 containing a nucleotide sequence in the vector were synthesized based on the nucleotide sequence of TPO gene [de Sauvage *et al.*, *Nature*, 369, 533 (1994)] and used as primers for the nucleotide sequence determination.

Determination of nucleotide sequence was carried out in accordance with the instructions attached to the kit and apparatus.

Of the above-mentioned plasmids, a plasmid pBS-TPO332 which coincided with the reported nucleotide sequence of the insertion fragment of TPO gene was used in the subsequent procedures.

2. Construction and expression of DNA which codes for TPO-ND28

Using the TPO-encoding DNA obtained in Example 1-1 and the ND28-encoding DNA obtained by the method described in Japanese Published Unexamined Patent Application No. 267292/88,

a fusion polypeptide of TPO and ND28 (TPO on the N-terminal side and ND28 on the C-terminal side), TPO-ND-28, was prepared in the following manner.

1) Construction of DNA (Sequence ID No. 5) which codes for TPO-ND28 (1) [Sequence ID No. 2; a type constructed through a linker (Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg; sequence ID No. 17)]

Though the mature type TPO comprises 332 amino acids, it is reported that its shortened protein consisting of its N-terminal side 153 amino acids can show the same activity of the complete length TPO [de Sauvage *et al.*, *Nature*, 369, 533 (1994)], so that a DNA which codes for TPO-ND28 (1) in which the 153 amino acids from the N-terminal of TPO, used as its N-terminal side, was fused with the complete length ND28 (174 amino acids) as the C-terminal side through a linker (Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg) was prepared in the following manner (cf. Fig. 1).

(i) Preparation of DNA which codes for the TPO moiety of TPO-ND28 (1)

In order to prepare a DNA which codes for the TPO moiety of TPO-ND28 (1) by means of PCR, a DNA primer having a nucleotide sequence (Sequence ID No. 18) which corresponds to the linker was synthesized as the 3' end primer (hereinafter referred to as "primer 3").

Using the thus synthesized primer 3 and the primer 1 and pBS-TPO332, PCR was carried out in the following manner.

A 0.1 ml portion of a reaction solution containing 10 ng of pBS-TPO332, 400 nM of the primer 3, 400 nM of the primer 1, 20 mM of Tris-HCl (pH 8.2), 10 mM of potassium chloride, 0.01 mg/ml of BSA, 2 mM of magnesium chloride, 6 mM of ammonium sulfate, 0.1% Triton X-100, 10% DMSO, 0.05 mM of dATP, 0.05 mM of dCTP, 0.05 mM of dGTP and 0.05 mM of dTTP was mixed with 2.5 units of Pfu polymerase to carry out PCR using PERKIN ELMER CETUS DNA Thermal Cycler (manufactured by Takara Shuzo Co., Ltd.) by 18 time repetition of a three step incubation at 94°C for 45 seconds, at 50°C for 1 minute and at 72°C for 1 minute.

The resulting reaction solution was subjected to phenol/chloroform extraction and ethanol precipitation, and the thus obtained precipitate was dissolved in 0.015 ml of TE buffer.

The thus prepared solution was mixed with restriction enzymes HindIII and XbaI to cleave the DNA amplified by PCR.

The resulting solution was subjected to an agarose gel electrophoresis, and a HindIII-XbaI treated DNA fragment of about 0.6 kb was isolated from the agarose gel.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the thus isolated DNA fragment (100 ng) was ligated with a HindIII-XbaI cleaved 2.9 kb fragment (50 ng) of pBlueScript II SK(-) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting

transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Nucleotide sequence of the insertion fragment in each plasmid was determined using Taq DyeDeoxy Terminator Cycle Sequencing Kit and ABI373A DNA Sequencer (manufactured by Applied Biosystems Japan Inc.). In determining the nucleotide sequence, primers having the nucleotide sequences of Sequence ID Nos. 9 to 12, 15 and 16 were used as primers for the nucleotide sequence determination.

Determination of nucleotide sequence was carried out in accordance with the instructions attached to the kit and apparatus.

Of the above-mentioned plasmids, plasmid pBS-T153LND which coincided with the reported nucleotide sequence of the insertion fragment of TPO gene was used in the subsequent procedures.

(ii) Preparation of DNA which codes for the ND28 moiety of TPO-ND28 (1)

In order to prepare a DNA which codes for the ND28 moiety of TPO-ND28 (1) by means of PCR, a primer having a nucleotide sequence (Sequence ID No. 19) which corresponds to the linker and the amino acid sequence of ND28 was synthesized as the 5′ end primer (hereinafter referred to as "primer 4"), and a primer having a nucleotide sequence (Sequence ID No. 20)

which corresponds to the C-terminal side amino acid sequence of ND28 was synthesized as the 3′ end primer (hereinafter referred to as "primer 5").

Using the thus synthesized primers and plasmid pCfBD28 (Japanese Published Unexamined Patent Application No. 267292/88), PCR was carried out in the following manner.

A 0.1 ml portion of a reaction solution containing 10 ng of pCfBD28, 400 nM of the primer 4, 400 nM of the primer 5, 20 mM of Tris-HCl (pH 8.2), 10 mM of potassium chloride, 0.01 mg/ml of BSA, 2 mM of magnesium chloride, 6 mM of ammonium sulfate, 0.1% Triton X-100, 10% DMSO, 0.05 mM of dATP, 0.05 mM of dCTP, 0.05 mM of dGTP and 0.05 mM of dTTP was mixed with 2.5 units of Pfu polymerase to carry out PCR using PERKIN ELMER CETUS DNA Thermal Cycler (manufactured by Takara Shuzo Co., Ltd.) by 18 time repetition of a three step incubation at 94°C for 45 seconds, at 50°C for 1 minute and at 72°C for 1 minute.

The resulting reaction solution was subjected to phenol/chloroform extraction and ethanol precipitation, and the thus obtained precipitate was dissolved in 0.015 ml of TE buffer.

The thus prepared solution was mixed with restriction enzymes SacII and XbaI to cleave the DNA amplified by PCR.

The resulting solution was subjected to an agarose gel electrophoresis, and a SacII-XbaI cleaved DNA fragment of about 0.5 kb was isolated from the agarose gel.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the thus isolated DNA fragment (100 ng) was ligated with a SacII-XbaI cleaved 2.9 kb fragment (50 ng) of pBlueScript II SK(-) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Nucleotide sequence of the insertion fragment in each plasmid was determined using Taq DyeDeoxy Terminator Cycle Sequencing Kit and ABI373A DNA Sequencer. In determining the nucleotide sequence, two DNA's having the nucleotide sequence of Sequence ID No. 21 or 22 containing a nucleotide sequence of the ND28-encoding DNA and two DNA's having the nucleotide sequence of Sequence ID No. 15 or 16 containing a sequence present in the vector were used as primers for the nucleotide sequence determination.

Determination of nucleotide sequence was carried out in accordance with the instructions attached to the kit and apparatus.

Of the above-mentioned plasmids, plasmid pBS-LND28 in which the nucleotide sequence of the insertion fragment

coincided with the nucleotide sequences of the ND28 gene and primers was used in the subsequent procedures.

(iii) Preparation of DNA which codes for TPO-ND28 (1)

The DNA's respectively which code for the TPO moiety and ND28 moiety prepared in Example 1-2-1)-(i) and (ii) were fused in the following manner.

A 2,000 ng portion of pBS-T153LND was cleaved with restriction enzymes SacII and XbaI and subjected to an agarose gel electrophoresis to isolate a DNA fragment of about 3.5 kb.

Also, a 500 ng portion of pBS-LND28 was cleaved with restriction enzymes SacII and XbaI and subjected to an agarose gel electrophoresis to isolate a DNA fragment of about 0.5 kb.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the DNA fragment of about 3.5 kb (100 ng) was ligated with the DNA fragment of about 0.5 kb (100 ng) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Structures of these plasmids were examined using restriction enzymes SacII and XbaI, and plasmid pBS-T153LND28 having a structure in which both of the DNA fragments are ligated with each other was used in the subsequent procedures.

2) Construction of DNA (Sequence ID No. 4) which codes for TPO-ND28 (2) [Sequence ID No. 1; a type constructed without a linker]

A DNA which codes for TPO-ND28 (2) in which the 154 amino acids of TPO from its N-terminal were fused with the N-terminal of D28 (174 amino acids) was prepared in the following manner (cf. Fig. 2).

(i) Preparation of DNA which codes for the TPO moiety of TPO-ND28 (2)

In order to prepare a DNA which codes for the TPO moiety of TPO-ND28 (2) by means of PCR, a primer having a nucleotide sequence shown in Sequence ID No. 23 which has a nucleotide sequence that corresponds to the amino acid sequences of TPO and ND28 was synthesized as the 3' side primer (hereinafter referred to as "primer 6").

Using the thus synthesized primer 6 and the primer 1 and pBS-TPO332, PCR was carried out in the following manner.

A 0.1 ml portion of a reaction solution containing 10 ng of pBS-TPO332, 400 nM of the primer 1, 400 nM of the primer 6, 20 mM of Tris-HCl (pH 8.2), 10 mM of potassium chloride, 0.01 mg/ml of BSA, 2 mM of magnesium chloride, 6 mM of ammonium sulfate, 0.1% Triton X-100, 10% DMSO, 0.05 mM of dATP, 0.05 mM of dCTP, 0.05 mM of dGTP and 0.05 mM of dTTP was mixed with 2.5 units of Pfu polymerase to carry out PCR using PERKIN ELMER CETUS DNA Thermal Cycler by 18 time repetition of a three step

incubatiion at 94°C for 45 seconds, at 50°C for 1 minute and at 72°C for 1 minute.

The resulting reaction solution was subjected to phenol/chloroform extraction and ethanol precipitation, and the thus obtained precipitate was dissolved in 0.015 ml of TE buffer.

The thus prepared solution was mixed with restriction enzymes HindIII and XhoI to cleave the DNA amplified by PCR.

The resulting solution was subjected to an agarose gel electrophoresis, and a HindIII-XhoI cleaved DNA fragment of about 0.5 kb was isolated from the agarose gel.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the thus isolated DNA fragment (100 ng) was ligated with a HindIII-XhoI cleaved 2.9 kb fragment (50 ng) of pBlueScript II SK(-) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Nucleotide sequence of the insertion fragment in each plasmid was determined using Taq DyeDeoxy Terminator Cycle Sequencing Kit and ABI373A DNA Sequencer (manufactured by Applied Biosystems Japan Inc.). In determining the nucleotide

sequence, primers having the nucleotide sequences of Sequence ID Nos. 9 to 12, 15 and 16 were used as primers for the nucleotide sequence determination.

Determination of nucleotide sequence was carried out in accordance with the instructions attached to the kit and apparatus.

Of the above-mentioned plasmids, plasmid pBS-T154ND in which the nucleotide sequence of the insertion fragment coincided with the nucleotide sequences of the TPO gene and primers was used in the subsequent procedures.

(ii) Preparation of DNA which codes for TPO-ND28 (2)

The DNA which codes for the TPO moiety prepared in Example 1-2-2)-(i) and the DNA which codes for the ND28 moiety prepared in Example 1-2-1)-(ii) were fused in the following manner.

A 200 ng portion of pBS-T154ND was cleaved with restriction enzymes KpnI and XhoI and subjected to agarose gel electrophoresis to isolate a DNA fragment of about 3.5 kb.

Also, a 500 ng portion of pBS-LND28 was cleaved with restriction enzymes KpnI and XhoI and subjected to agarose gel electrophoresis to isolate a DNA fragment of about 0.5 kb.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the DNA fragment of about 3.5 kb (100 ng) was ligated with the DNA fragment of about 0.5 kb (100 ng) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Structures of these plasmids were examined using restriction enzymes KpnII and XhoI, and plasmid pBS-T154ND28 having a structure in which both of the DNA fragments are ligated with each other was used in the subsequent procedures.

3) Construction of DNA (Sequence ID No. 6) which codes for TPO-ND28 (3) [Sequence ID No. 3; a type constructed through a linker (Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg; sequence ID No. 24)]

A DNA which codes for TPO-ND28 (3) in which the 153 amino acids from the N-terminal of TPO, used as its N-terminal side, was fused with the complete length ND28 (174 amino acids) as the C-terminal side through a linker (Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg) was prepared in the following manner (cf. Fig. 3).

In order to ligate the DNA which codes for the TPO moiety prepared in Example 1-2-2)-(i) with the DNA which codes for the ND28 moiety prepared in Example 1-2-1)-(ii) through a linker (Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg), two DNA's shown in Sequence ID Nos. 25 and 26 having nucleotide sequences which form SplI-BbeI complementary

termini on both sides corresponding to the amino acid sequences of linkers were synthesized.

A 0.02 ml portion of a solution containing 0.01 mM of the DNA shown in Sequence ID No. 25, 5 mM of ATP, 50 mM of Tris-HCl (pH 8.0), 10 mM of magnesium chloride and 5 mM of dithiothreitol was mixed with 10 units of T4 Polynucleotide Kinase (manufactured by Takara Shuzo Co., Ltd.), and the mixture was allowed to stand at 37°C for 30 minutes and then heated at 70°C for 3 minutes to obtain treating solution (1).

The DNA shown in Sequence ID No. 26 was also treated in the same manner to obtain treating solution (2).

Treating solution (1) was mixed with treating solution (2), and the mixture was incubated at 90°C for 5 minutes and then gradually cooled to 22°C spending 3 hours to prepare double-stranded DNA.

The thus prepared double-stranded DNA was inserted into the connecting site of the TPO-coding gene and ND28-coding gene of pBS-T154ND28 obtained in Example 1-2-2)-(ii) in the following manner.

A 2,000 ng portion of pBS-T154ND28 was cleaved with restriction enzymes BbeI and SplI and subjected to an agarose gel electrophoresis to isolate a DNA fragment of about 4.0 kb.

Using DNA Ligation Kit Ver. 1 (manufactured by Takara Shuzo Co., Ltd.), the DNA fragment of about 4.0 kb (100 ng) was ligated with the above-mentioned double-stranded DNA (12.5 pmole) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Nucleotide sequence of the insertion fragment in each plasmid was determined using Taq DyeDeoxy Terminator Cycle Sequencing Kit and ABI373A DNA Sequencer. In determining the nucleotide sequence, two DNA's shown in Sequence ID Nos. 12 and 22 were used as primers. Determination of nucleotide sequence was carried out in accordance with the instructions attached to the kit and apparatus.

Of these plasmids, plasmid named pBS-T153ND28LN1 in which the nucleotide sequence of the insertion fragment coincided with the nucleotide sequence of the linker DNA was used in the subsequent procedures.

Example 2 Production of TPO-CSF

The TPO-CSF was produced by effecting expression of the DNA which codes for the TPO-CSF in animal cells in the following manner.

1) Production of TPO-ND28 (1) and TPO-ND28 (2)

Plasmid pcDNA3 (manufactured by Invitrogen Co.) was cleaved with EcoRI and NotI and subjected to an agarose gel

electrophoresis to isolate a DNA fragment (vector side) of about 5.4 kb.

Also, pBS-T153LND28 and pBS-T154ND28 obtained in Example 1-2-1)-(iii) and Example 1-2-2)-(ii) were separately cleaved with EcoRI and NotI and subjected to agarose gel electrophoresis to isolate a DNA fragment (insert side) of about 1.1 kb from each plasmid.

Using DNA Ligation Kit Ver. 1, the vector side DNA fragment of about 5.4 kb (100 ng) was ligated with each of the insert side DNA fragments (100 ng) (volume of the reaction solution: 0.018 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Structure of each plasmid was examined using restriction enzymes EcoRI and NotI to select plasmids containing respective inserts having a structure in which the vector side and insert side DNA fragments are ligated with each other, and plasmid pCD-153LND28 containing a TPO-ND28 (1) encoding gene and plasmid pCD-154ND28 containing a TPO-ND28 (2) encoding gene were used in the subsequent procedure.

Plasmid pCD-153LND28 or pCD-154ND28 was introduced into animal cells by electroporation [Potter *et al.*, *Proc. Natl.*

Acad. Sci., USA, 81, 7161 (1984)] and its expression was effected in the following manner.

COS 7 cells were cultivated in D-MEM medium (manufactured by GibcoBRL Co., product No. 11885-50) which was further supplemented with 10% fetal bovine serum.

The COS 7 cells obtained by cultivation were suspended in K-PBS buffer (137 mM potassium chloride, 2.7 mM sodium chloride, 8.1 mM disodium hydrogenphosphate, 1.5 mM sodium dihydrogenphosphate, 4 mM magnesium chloride) to prepare a cell suspension of 8×10^8 cells/ml.

A 0.2 ml portion of the cell suspension was injected into a Pulser Cuvette (manufactured by BIO RAD LABORATORIES) having a slit width of 0.2 cm.

A 4 μg portion of pCD-153LND28 or pCD-154ND28 was added to the cuvette, thoroughly mixed with the suspension and then subjected to pulse application using an electroporation apparatus (Gene Pulser, manufactured by BIO RAD LABORATORIES) under conditions of 200 Ω, 0.3 kv/cm and 0.125 mF.

The pulse-treated solution was allowed to stand in ice for 5 minutes, suspended in 10 ml of D-MEM medium supplemented with 10% fetal bovine serum and then cultivated at 37°C for 72 hours in a CO_2 incubator.

The culture broth was subjected to centrifugation, and the resulting culture supernatant was filtered through a filter of 220 nm pore size to obtain a solution of TPO-ND28 (1) or TPO-ND28 (2).

2) Production of TPO-ND28 (3)

A plasmid PAGE210 was used as the vector for use in the expression of TPO-ND28 (3). The vector pAGE210 is a derivative of pAGE248 [Sasaki *et al.*, *J. Biol. Chem.*, 269, 14730, (1994)], in which the Moloney murine leukemia virus promoter (XhoI-HindIII fragment) has been replaced by SV40 early promoter (XhoI-HindIII fragment) of pAGE103 [Mizukami *et al.*, *J. Biochem.*, 101, 1307 (1987)].

Plasmid pAGE210 was cleaved with KpnI and HindIII and subjected to an agarose gel electrophoresis to isolate a DNA fragment (vector side) of about 9.0 kb.

Separately from this, pBS-TPO322 obtained in Example 1-1 was cleaved with KpnI and HindIII, and pBS-153ND28LN1 obtained in Example 1-2-3) was cleaved with KpnI and then partially with HindIII, and each of the resulting cleaved fragments was subjected to an agarose gel electrophoresis to isolate a DNA fragment (insert side) of about 1.1 kb from each plasmid.

Using DNA Ligation Kit Ver. 1, the vector side DNA fragment of about 9.0 kb (100 ng) was ligated with each of the insert side DNA fragments of about 1.1 kb (100 ng) (volume of the reaction solution: 0.012 ml).

Using this reaction solution, the *Escherichia coli* strain DH5α was transformed in the usual way, and the resulting transformant was spread on LB agar medium containing 50 μg/ml of ampicillin and cultivated overnight at 37°C.

Plasmids were isolated from several transformant strains grown on the medium in accordance with a known method.

Structure of each plasmid was examined using a restriction enzyme KpnI to select plasmids containing respective inserts having a structure in which the vector side and insert side DNA fragments are ligated with each other, and plasmid pAGE210-T332 containing TPO encoding gene and plasmid pAGE210-LN1 containing TPO-ND28 (3) encoding gene were used in the subsequent procedure.

Plasmid pAGE210-T332 or pAGE210-LN1 was introduced into animal cells by electroporation.

CHO cells were cultivated in MEM medium (1) (manufactured by GibcoBRL Co., product No. 19000-024) which was further supplemented with 10% fetal bovine serum.

The CHO cells obtained by cultivation were suspended in K-PBS buffer to prepare a cell suspension of 8×10^6 cells/ml.

A 0.2 ml portion of the cell suspension was injected into Pulser Cuvette having a slit width of 0.2 cm.

A 4 μg portion of pAGE210-T332 or pAGE210-LN1 was added to the cuvette, thoroughly mixed with the suspension and then subjected to pulse application using an electroporation apparatus, Gene Pulser, under conditions of 0.35 kv/cm and 0.25 mF.

The pulse-treated solution was allowed to stand in ice for 5 minutes, suspended in 10 ml of MEM medium supplemented

with 10% fetal bovine serum and then cultivated at 37°C for 24 hours in a CO_2 incubator.

The thus cultivated cells were again cultivated for 2 weeks in MEM medium (1) supplemented with 10% fetal bovine serum and 0.3 mg/ml of hygromycin.

The resulting cells were further cultivated for 2 weeks in MEM medium (2) (manufactured by GibcoBRL Co., code No. 12000-022) supplemented with 10% fetal bovine serum and 50 nM methotrexate (hereinafter referred to as MTX).

The cultivation was repeated in the same manner by successively increasing the MTX concentration to 100 nM, 500 nM and 1,000 nM in that order, thereby obtaining strains resistant to 1,000 nM TMX.

Each of the 1,000 nM MTX resistant strains was grown in MEM medium (2) supplemented with 10% fetal bovine serum, the medium was exchanged with a serum-free medium for CHO cell use, CHO-S-SFMII (manufactured by GibcoBRL Co., code No. 12052-015), and then the strain was cultivated again for 96 to 144 hours.

By subjecting the culture broth to centrifugation, a culture supernatant containing TPO or TPO-ND28 (3) was obtained.

Example 3 Purification of TPO-ND28 (3) and TPO

A 1,000 ml portion of TPO-ND28 (3) or TPO obtained in Example 2-2) was concentrated to 50 ml using Centriprep

(manufactured by Amicon Co.) to prepare a concentrated solution.

A 50 ml portion of each of the concentrated solutions was applied to XK50 column (manufactured by Pharmacia K.K.) which has been packed with 1,000 ml of Sephacryl S-200 resin (manufactured by Pharmacia K.K.) and filled with a phosphate buffer (9.4 mM sodium phosphate (pH 7.2), 137 mM NaCl, 2.7 mM KCl).

Elution of TPO-ND28 (3) or TPO was effected by passing the phosphate buffer through the column at a flow rate of 3 ml/minute.

The eluates were pooled for every 12.5 minutes, and the resulting fractions were checked for their TPO and G-CSF activities by an MTT assay method which will be described later, thereby obtaining purified TPO-ND28 (3) or TPO.

Example 4 Modification of TPO-ND28 (3) with polyethylene glycol

To ice-cooled water was added 20 kd PEG-succinimidyl propionate (manufactured by Shearwater Polymers Co.) to a final concentration of 400 mg/ml.

A 50 µl portion of the thus prepared aqueous solution was mixed with 200 µl of the TPO-ND28 (3) solution obtained in Example 3 and 150 µl of distilled water. The mixture was allowed to stand for 12 hours at 4°C, thereby effecting modification of TPO-ND28 (3) by polyethylene glycol.

The TPO-ND28 (3) thus modified with polyethylene glycol (hereinafter referred to as PEG-TPO-ND28 (3)) was applied to a column of Super Rose 610/30 (manufactured by Pharmacia K.K.) which has been filled in advance with a phosphate buffer (9.4 mM sodium phosphate (pH 7.2), 137 mM NaCl, 2.7 mM KCl).

Elution was effected by passing the phosphate buffer through the column at a flow rate of 0.5 ml/minute.

The eluates were pooled for every 1 minute, and the resulting fractions were checked for their G-CSF and TPO activities by MTT assay method which will be described later.

The results are shown in Table 5.

The G-CSF and TPO activities originated from un-modified TPO-ND28 (3) were detected 34 to 40 minutes after

TABLE 5

Elution time (minutes)	0	10	14	16	18	20	22	24	26	28	30	32	34	36	38	40
G-CSF activity	-	-	-	+	+	±	+	+	+	+	-	-	+	+	+	+
TPO activity	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+

-: no activity; +: activity

Test Example 1 Measurement of TPO-ND28 molecular weight

Using the TPO-ND28 (1) solution obtained in Example 2-1), its molecular weight was measured by a gel filtration chromatography in the following manner.

A 0.2 ml portion of the TPO-ND28 (1) solution was applied to a column of Super Rose 610/30 (manufactured by Pharmacia K.K.) which has been equilibrated in advance with a phosphate buffer (9.4 mM sodium phosphate (pH 7.2), 137 mM NaCl, 2.7 mM KCl), and elution of TPO-ND28 (1) was effected by passing the phosphate buffer through the column at a flow rate of 0.5 ml/minute.

The eluates were pooled for every 0.5 minute, and the resulting fractions were checked for their TPO and G-CSF activities by an MTT assay method which will be described later.

Table 6 shows elution time from Super Rose and measured values of TPO and G-CSF activities.

The TPO and G-CSF activities reached the maximum after 33.5 minutes of the elution.

Separately from this, thyroglobulin (molecular weight: 670,000), aldolase (molecular weight: 160,000), bovine serum albumin (molecular weight: 69,000) and G-CSF (molecular weight: 20,000) were used as the standard molecular weight proteins and passed through Super Rose to obtain relationship between elution time and molecular weight.

Molecular weight of TPO-ND28 (1) deduced from the 33.5 minutes of elution time was about 40,000.

TABLE 6

Elution time	0	20	30	32	33	33.5	34	35	37	42
TPO activity (A_{540})	0.08	0.07	0.08	0.19	0.30	0.32	0.29	0.18	0.09	0.08
G-CSF activity (A_{540})	0.00	0.00	0.03	0.14	0.29	0.32	0.30	0.22	0.05	0.00

Test Example 2 Biological activity of TPO-CSF

Basic construction for the measurement of the cell growth-stimulating activity of a solution to be tested (TPO-ND28 solution) upon cells to be tested is as follows.

Each solution to be tested (TPO-ND28 solution), TPO standard solution and ND28 standard solution is made into 10-fold serial dilutions, and a 0.01 ml portion of each of the dilutions is added to each well of a microtiter plate.

Actively growing cells to be tested are collected from a culture broth by centrifugation, washed and then re-suspended

in a medium for testing use to a most suitable cell density for each testing.

The thus prepared cell suspension is dispensed in 0.09 ml portions into wells of the above-mentioned microtiter plate which has been prepared by dispensing dilutions of the solution to be tested, TPO standard solution or ND28 standard solution in 0.01 ml portions.

The microtiter plate is incubated at 37°C in a completely moist 5% CO_2 incubator and then used in the following testing.

A 0.01 ml portion of 0.5 mg/ml solution of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide] is added to each well, incubated for 4 hours, mixed with 0.15 ml of 0.1 N hydrochloric acid/isopropyl alcohol solution and then stirred to extract pigment from the cells, subsequently judging growth of the cells by measuring the amount of the pigment by its absorbance at 540 nm.

This method for the measurement of cell growth-stimulating activity is hereinafter called the MTT assay.

(1) Measurement of cell growth-stimulating activity upon Ba/F3 cells

The Ba/F3 cells which grow depending on the presence of mouse IL-3 were cultivated in Iscove's modified Dulbecco medium (hereinafter referred to as "IMDM") which has been supplemented with 10% heat-inactivated fetal calf serum (hereinafter

referred to as "FCS") and mouse IL-3 (culture supernatant of WEHI-3B).

Using the thus cultivated Ba/F3 cells, the cell growth-stimulating activity was measured by the MTT assay using the just described medium but in the absence of mouse IL-3.

The MTT assay was carried out with an inoculation density of 10,000 cells per well and by incubating the plate in 5% CO_2 for 48 hours.

Results of the MTT assay showed that each of TPO, ND28 and TPO-ND28 (1), (2) and (3) had no Ba/F3 cell growth-stimulating activity.

(2) Measurement of cell growth-stimulating activity upon Ba/F3-cmpl

The Ba/F3-cmpl cells which grow depending on the presence of mouse IL-3 or TPO were cultivated in IMDM which has been supplemented with 10% heat-inactivated FCS, 0.5 mg/ml of G418 and mouse IL-3 (culture supernatant of WEHI-3B).

Using the thus cultivated Ba/F3-cmpl cells, the cell growth-stimulating activity was measured by MTT assay using the just described medium but in the absence of mouse IL-3.

The MTT assay was carried out with an inoculation density of 10,000 cells per well and by incubating the plate in 5% CO_2 for 48 hours.

Results of the MTT assay showed that each of TPO and TPO-ND28 (1), (2) and (3) had Ba/F3-cmp cell growth-stimulating activity.

(3) Measurement of cell growth-stimulating activity upon NFS-60 cells

The NFS-60 cells which grow depending on the presence of human G-CSF or mouse IL-3 were cultivated in RPMI medium which has been supplemented with 10% heat-inactivated FCS, 2 mM glutamine, P/S (100 U/ml of penicillin, 100 mg/ml of streptomycin) and 1.0 ng/ml of recombinant type human G-CSF.

Using the thus cultivated NFS-60 cells, the cell growth-stimulating activity was measured by the MTT assay using the just described medium but in the absence of G-CSF.

The MTT assay was carried out with an inoculation density of 10,000 cells per well and by incubating the plate in 5% CO_2 for 48 hours.

Results of the MTT assay showed that each of ND28 and TPO-ND28 (1), (2) and (3) had NFS-60 cell growth-stimulating activity.

Test Example 3 Effect of TPO-ND28 on mouse myeloid cells

A BALB/c mouse of 8 weeks of age was sacrificed to excise the femur and tibia system whose both ends were subsequently cut with scissors. The needle of a syringe filled with RPMI solution containing 10% FCS was inserted into the section of femur and tibis to blow off myeloid cells into a small test tube, and the cells were allowed to stand for 5 minutes.

Using a Pasteur pipette, the supernatant fluid in the test tube was drawn up taking care not to contaminate it with

the precipitate, and the supernatant fluid was overlaid on Nycoprep 1.077 Animal (manufactured by NYCOMED Co., product No. 1002380) and subjected to 15 minutes of centrifugation at 600 g to isolate mouse mono nuclear cells (hereinafter referred to as "MNC").

The MNC were made into a suspension of 5×10^5 cells/ml with a solution containing a solution to be tested, 10% FCS, 1% BSA and 0.6 mg/ml of transferrin (manufactured by Boehringer Manheim Co.) and cultivated for 5 days in a CO_2 incubator (BNA-120D, manufactured by TABAI Co.) under conditions of 37°C, 5% CO_2 and 95% or more of humidity.

As the solution to be tested, a solution of TPO, ND28 or TPO-ND28 having a final concentration of 1.0, 10 or 100 ng/ml or a solution in which the same volume of TPO and ND-28 solutions having the above-mentioned concentration were mixed (TPO/ND28) was used. The TPO and ND28 obtained in Example 3 were used.

After completion of the cultivation, conditions of the differentiation of MNC were examined by measuring the amount of CD61 expressed which is an index of differentiation into megakaryocyte system [*J. Med.*, 311, 1084 (1984)] and the amount of Gr-1 expressed which is an index of differentiation into the granulocyte system [*J. Immunol.*, 144, 22 (1991)].

After staining with anti mouse CD61-FITC monoclonal antibody (manufactured by PHARMINGEN Co., product No. 01864D) and anti mouse Gr-1-PE monoclonal antibody (manufactured by

PHARMINGEN Co., product No. 01215A), expressed amounts of CD61 and Gr-1 were measured using an ELITE flow cytometer (manufactured by Coulter Co.).

The results are shown in Table 7.

TABLE 7

Solution to be tested	Concentration (ng/ml)	Expressed cells (%) Gr-1	CD61
no addition		1.0	1.0
ND28	1.0	49.1	7.6
	10.0	40.7	4.9
	100.0	44.5	4.6
TPO	1.0	36.7	8.7
	10.0	37.7	17.8
	100.0	37.1	21.9
TPO/ND28	1.0	50.7	10.3
	10.0	40.6	10.4
	100.0	49.2	5.7
TPO-ND28	1.0	50.5	22.1
	10.0	49.8	26.6
	100.0	41.0	18.8

When the solution to be tested prepared by mixing the same amount of TPO and ND28 (TPO/ND28) was added, Gr-1 expressed cells were generated in a level similar to the case

of the addition of the solution to be tested containing ND28 alone, thus showing differentiation of MNC into the granulocyte system, but frequency of the generation of CD61 expressed cells was lower than the case of the addition of the solution to be tested containing TPO alone, thus showing decreased differentiation into the megakaryocyte system. These results suggest that, when the same amount of TPO and ND28 are present, MNC reacts mostly with ND28 and differentiates into the granulocyte system.

However, when the fusion polypeptide of TPO and ND28, namely TPO-ND28, was added as the solution to be tested, frequency of the generation of CD61 expressed cells was similar to or higher than the case of the addition of the solution to be tested containing TPO alone and two times or more higher than the case of the addition of TPO/ND28. What is more, the frequency of the generation of Gr-1 expressed cells was also similar to the case of the addition of the solution to be tested containing ND28 alone.

Test Example 4 Platelet and leukocyte production-enhancing function in mice

A 10μg/ml solution of TPO or a 10 μg/ml solution of TPO-ND28 (3) obtained in Example 3 was administered by subcutaneous injection to BALB/c mice (males, 7 weeks of age) with a dose of 0.2 ml per 20 g body weight of each mouse, once a day continuously for 4 days starting on the first day of the test (treated groups, 4 animals per one group). A blood sample

was collected from the ophthalmic vein of each animal on the fifth day of the test to count the number of platelets and leukocytes by a microcell counter (Sysmex F800, manufactured by Toa Iyo Denshi Co.).

After introducing the plasmid pAGE210 used for the expression of TPO or TPO-ND28 (3) gene into CHO cells in accordance with the method described in Example 2-2), the cells were cultivated, the resulting culture supernatant was treated by the same TPO-ND28 (3) purification procedure described in Example 3, and an elution fraction corresponding to the elution fraction of TPO-ND28 (3) was used as a blank solution to count the number of platelets and leukocytes by the above-mentioned method.

In order to compare and examine effects of TPO and TPO-ND28 (3), the increasing ratio (%) of the number of platelets and leukocytes in the group in which each of these substances were administered to that in the blank solution-administered group was calculated based on the following formula:

[platelet or leukocyte counts in mice of TPO- or TPO-ND28 (3)-administered group]/[platelet or leukocyte count in mice of blank solution-administered group] x 100

The results are shown in Table 8.

TABLE 8

Test substance	Increasing ratio of platelets (%)	Increasing ratio of leukocytes (%)
TPO	219	106
TPO-ND28	170	160

INDUSTRIAL APPLICABILITY

A fusion polypeptide comprising a polypeptide having both G-CSF activity and a polypeptide having TPO activity is provided by the present invention. The fusion polypeptide of the present invention can form and amplify platelets and leukocytes simultaneously and can control formation of megakaryocyte colonies and neutrophil colonies and differentiation or maturation of megakaryocyte precursors and neutrophil precursors.

SEQUENCE LISTING

Sequence ID No.: 1

Sequence Length: 328

Sequence Type: amino acid

Strandedness: single

Topology: linear

Molecular Type: peptide

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation:

Location: 1..154

Designation:

Location: 154..328

Sequence

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Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
 1               5                  10                  15
Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
            20                  25                  30
His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
        35                  40                  45
Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
    50                  55                  60
Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65                  70                  75                  80
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Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
85 90 95
Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
100 105 110
Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
115 120 125
Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
130 135 140
Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala Pro Thr Tyr Arg Ala
145 150 155 160
Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg
165 170 175
Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr
180 185 190
Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu
195 200 205
Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln
210 215 220
Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln
225 230 235 240
Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr
245 250 255
Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp
260 265 270
Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln
275 280 285

Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
290 295 300
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
305 310 315 320
Val Leu Arg His Leu Ala Gln Pro
325

Sequence ID No.: 2

Sequence Length: 340

Sequence Type: amino acid

Strandedness: single

Topology: linear

Molecular Type: peptide

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation:

Location: 1..153

Designation:

Location: 167..340

Sequence

Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
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Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
20 25 30

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His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
          35                  40                  45
Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
      50                  55                  60
Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
 65                  70                  75                  80
Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
                 85                  90                  95
Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
            100                 105                 110
Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
        115                 120                 125
Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
    130                 135                 140
Val Gly Gly Ser Thr Leu Cys Val Arg Gly Gly Gly Ser Gly Gly Gly
145                 150                 155                 160
Ser Gly Gly Gly Ser Arg Ala Pro Thr Tyr Arg Ala Ser Ser Leu Pro
                165                 170                 175
Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg Lys Ile Gln Gly
            180                 185                 190
Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys
        195                 200                 205
His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp
    210                 215                 220
Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys
225                 230                 235                 240
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Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln
245 250 255

Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu
260 265 270

Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu
275 280 285

Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro
290 295 300

Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala
305 310 315 320

Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His
325 330 335

Leu Ala Gln Pro
340

Sequence ID No.: 3

Sequence Length: 344

Sequence Type: amino acid

Strandedness: single

Topology: linear

Molecular Type: peptide

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation:

Location: 1..153

Designation:

Location: 171..344

Sequence

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Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
 1               5                  10                  15
Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
            20                  25                  30
His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
        35                  40                  45
Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
    50                  55                  60
Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65                  70                  75                  80
Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
                85                  90                  95
Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
            100                 105                 110
Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
        115                 120                 125
Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
    130                 135                 140
Val Gly Gly Ser Thr Leu Cys Val Arg Ser Gly Gly Gly Ser Gly Gly
145                 150                 155                 160
Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg Ala Pro Thr Tyr Arg Ala
                165                 170                 175
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Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg
180 185 190
Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr
195 200 205
Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu
210 215 220
Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln
225 230 235 240
Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln
245 250 255
Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr
260 265 270
Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp
275 280 285
Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln
290 295 300
Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
305 310 315 320
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
325 330 335
Val Leu Arg His Leu Ala Gln Pro
340

Sequence ID No.: 4

Sequence Length: 1047

Sequence Type: nucleic acid

Strandedness: double

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation: sig peptide

Location: 1..63

Designation: CDS

Location: 64..1047

Sequence

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ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA   48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
    -20                 -15                 -10

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC   96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                  1                   5                  10

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC  144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
            15                  20                  25

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT  192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
        30                  35                  40
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GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG  240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55
GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG  288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75
GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG  336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90
CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC  384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                 100                 105
CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT  432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
        110                 115                 120
CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG  480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135
CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTA CGG CGG GCG  528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala
140                 145                 150                 155
CCA ACA TAT CGC GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC  576
Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
                160                 165                 170
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TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG 624
Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
175 180 185
AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG 672
Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
190 195 200
CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC 720
Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
205 210 215
AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC 768
Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
220 225 230 235
CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC 816
Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
240 245 250
GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT 864
Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
255 260 265
GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC 912
Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
270 275 280
CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG 960
Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
285 290 295

CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG 1008
Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
300 305 310 315
GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC 1047
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
320 325

Sequence ID No.: 5

Sequence Length: 1083

Sequence Type: nucleic acid

Strandedness: double

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation: sig peptide

Location: 1..63

Designation: CDS

Location: 64..1083

Sequence

ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA 48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-20 -15 -10

```
AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC   96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                   1                   5                  10
CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC  144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25
CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT  192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40
GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG  240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55
GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG  288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75
GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG  336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90
CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC  384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                  100                 105
CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT  432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
         110                 115                 120
```

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CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG  480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135
CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTC AGG GGT GGC  528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Gly Gly
140                 145                 150                 155
GGT TCT GGA GGT GGT TCC GGA GGG GGT TCT AGA GCA CCA ACA TAT CGC  576
Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg Ala Pro Thr Tyr Arg
                160                 165                 170
GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC TTA GAG CAA GTG  624
Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val
            175                 180                 185
AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG AAG CTG TGT GCC  672
Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala
        190                 195                 200
ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT  720
Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser
    205                 210                 215
CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC AGC CAG GCC CTG  768
Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu
220                 225                 230                 235
CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC  816
Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr
                240                 245                 250
```

CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC GAG TTG GGT CCC 864
Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro
255 260 265
ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC 912
Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile
270 275 280
TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC 960
Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr
285 290 295
CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA 1008
Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly
300 305 310 315
GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC 1056
Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr
320 325 330
CGC GTT CTA CGC CAC CTT GCC CAG CCC 1083
Arg Val Leu Arg His Leu Ala Gln Pro
335 340

Sequence ID No.: 6

Sequence Length: 1095

Sequence Type: nucleic acid

Strandedness: double

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Original Source

Organism: human (Homo sapiens)

Sequence Characteristics:

Designation: sig peptide

Location: 1..63

Designation: CDS

Location: 64..1095

Sequence

```
ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA   48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
    -20                 -15                 -10

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC   96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                  1                   5                  10

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC  144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT  192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG  240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG  288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75
```

GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG 336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
80 85 90

CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC 384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
95 100 105

CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT 432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
110 115 120

CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG 480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
125 130 135

CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTA CGG TCC GGA 528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Ser Gly
140 145 150 155

GGT GGC TCT GGC GGT GGT TCT GGT GGC GGC TCC GGA GGC GGT CGT GCG 576
Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg Ala
160 165 170

CCA ACA TAT CGC GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC 624
Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
175 180 185

TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG 672
Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
190 195 200

```
AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG  720
Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
    205                 210                 215
CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC  768
Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
220                 225                 230                 235
AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC  816
Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
                240                 245                 250
CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC  864
Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
            255                 260                 265
GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT  912
Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
        270                 275                 280
GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC  960
Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
    285                 290                 295
CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG 1008
Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
300                 305                 310                 315
CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG 1056
Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
                320                 325                 330
```

GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC 1095
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
335 340

Sequence ID No.: 7

Sequence Length: 44

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: sig peptide

Location: 27..44

Sequence

CTCTCCAAGC TTGAATTCCG GCCAGAATGG AGCTGACTGA ATTG 44

Sequence ID No.: 8

Sequence Length: 47

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 23..47

Sequence

GTAGAGGTAC CGCGGCCGCT TACCCTTCCT GAGACAGATT CTGGGAG 47

Sequence ID No.: 9

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

TGAACCTCTG GGCACTGGCT CAGT 24

Sequence ID No.: 10

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

GCTGCCTGCT GTGGACTTTA GCTT 24

Sequence ID No.: 11

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

TGTTGGAAGC TCAGGAAGAT GGCA 24

Sequence ID No.: 12

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

CCTGATGCTT GTAGGAGGGT CCAC 24

Sequence ID No.: 13

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

TCAAGAGTTC GTGTATCCTG TTCA 24

Sequence ID No.: 14

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

GAATGGAACT CGTGGACTCT TTCC 24

Sequence ID No.: 15

Sequence Length: 17

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence

GTAAAACGAC GGCCAGT 17

Sequence ID No.: 16

Sequence Length: 17

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence

CAGGAAACAG CTATGAC 17

Sequence ID No.: 17

Sequence Length: 13

Sequence Type: amino acid

Strandedness: single

Topology: linear

Molecular Type: peptide

Sequence

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg

1 5 10

Sequence ID No.: 18

Sequence Length: 66

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..3

Designation: CDS

Location: 43..66

Sequence

TGCTCTAGAA CCGCCTCCGG AACCACCTCC AGAACCGCCA CCCCTGACGC AGAGGGTGGA 60

CCCTCC 66

Sequence ID No.: 19

Sequence Length: 45

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 22..45

Sequence

GGTTCCGGAG GCGGTTCTAG AGCACCAACA TATCGCGCCT CGAGT 45

Sequence ID No.: 20

Sequence Length: 48

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 28..48

Sequence

CATTCCGCGG GGTACCGCGG CCGCTCAGGG CTGGGCAAGG TGGCGTAG 48

Sequence ID No.: 21

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

GGCTGCTTGA GCCAACTCCA TAGC 24

Sequence ID No.: 22

Sequence Length: 24

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..24

Sequence

GACCCAACTC GGGGGAGATC CCTT 24

Sequence ID No.: 23

Sequence Length: 57

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS

Location: 1..27

Designation: CDS

Location: 28..57

Designation: mutation

Location: 25

Designation: mutation

Location: 33..34

Sequence

TAGACTCGAG GCGCGATATG TTGGCGCCCG CCGTACGCAG AGGGTGGACC CTCCTAC 57

Sequence ID No.: 24

Sequence Length: 17

Sequence Type: amino acid

Strandedness: single

Topology: linear

Molecular Type: peptide

Sequence

Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg
1 5 10 15

Sequence ID No.: 25

Sequence Length: 61

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS of TPO

Location: 1..6

Designation: linker peptide

Location: 7..57

Designation: CDS of ND28

Location: 58..61

Designation: SplI

Location: 1..5

Designation: MroI

Location: 7..12

Designation: MroI

Location: 43..48

Designation: BbeI

Location: 58..61

Designation: mutation

Location: 4..5

Sequence

GTACGGTCCG GAGGTGGCTC TGGCGGTGGT TCTGGTGGCG GCTCCGGAGG CGGTCGTGCG C 61

Sequence ID No.: 26

Sequence Length: 53

Sequence Type: nucleic acid

Strandedness: single

Topology: linear

Molecular Type: other nucleic acid, synthetic DNA

Sequence Characteristics:

Designation: CDS of TPO

Location: 52..53

Designation: linker peptide

Location: 1..51

Designation: SplI

Location: 53

Designation: MroI

Location: 10..15

Designation: MroI

Location: 46..51

Sequence

ACGACCGCCT CCGGAGCCGC CACCAGAACC ACCGCCAGAG CCACCTCCGG ACC 53

CLAIMS

1. A fusion polypeptide which comprises a polypeptide having a human granulocyte colony stimulating factor activity and a polypeptide having a platelet growth factor activity.

2. A fusion polypeptide in which a polypeptide having a human granulocyte colony stimulating factor activity and a polypeptide having a platelet growth factor activity are fused via a spacer peptide.

3. The fusion polypeptide according to claim 1 wherein the fusion polypeptide is a polypeptide which contains an amino acid sequence selected from the amino acid sequences shown in Sequence ID Nos. 1, 2, 3, 4, 5 and 6.

4. A fusion polypeptide in which one or more amino acids are added, deleted or substituted in the amino acid sequence of the fusion polypeptide disclosed in claim 1, 2 or 3, and which has a human granulocyte colony stimulating factor activity and a platelet growth factor activity.

5. A fusion polypeptide having a human granulocyte colony stimulating factor activity and a platelet growth factor activity in which at least one amino group of the fusion polypeptide disclosed in claim 1, 2, 3 or 4 is chemically modified with a polyalkylene glycol derivative.

6. The fusion polypeptide according to claim 5 wherein the polyalkylene glycol derivative is a polyethylene glycol derivative, a polypropylene glycol derivative or a polyoxyethylene-polyoxypropylene copolymer derivative.

7. A DNA which codes for the fusion polypeptide disclosed in claim 1, 2, 3 or 4.

8. The DNA according to claim 6 wherein the DNA is a DNA which contains a sequence selected from the DNA sequences shown in Sequence ID Nos. 4, 5 and 6.

9. An anemia-treating composition containing the fusion polypeptide disclosed in claim 1, 2, 3, 4 or 5 as an active ingredient.

ABSTRACT

The present invention relates to a fusion polypeptide which comprises a polypeptide having G-CSF activity and a polypeptide having TPO activity and DNA which codes for the fusion polypeptide, to a fusion polypeptide in which a polypeptide having G-CSF activity and a polypeptide having TPO activity are fused via a spacer peptide and DNA which codes for the fusion polypeptide and to a polypeptide in which the fusion polypeptide comprising a polypeptide having G-CSF activity and a polypeptide having TPO activity is chemically modified with a polyalkylene glycol derivative. It also relates to an anemia-treating composition containing the fusion polypeptide as an active ingredient.

FIG. 1

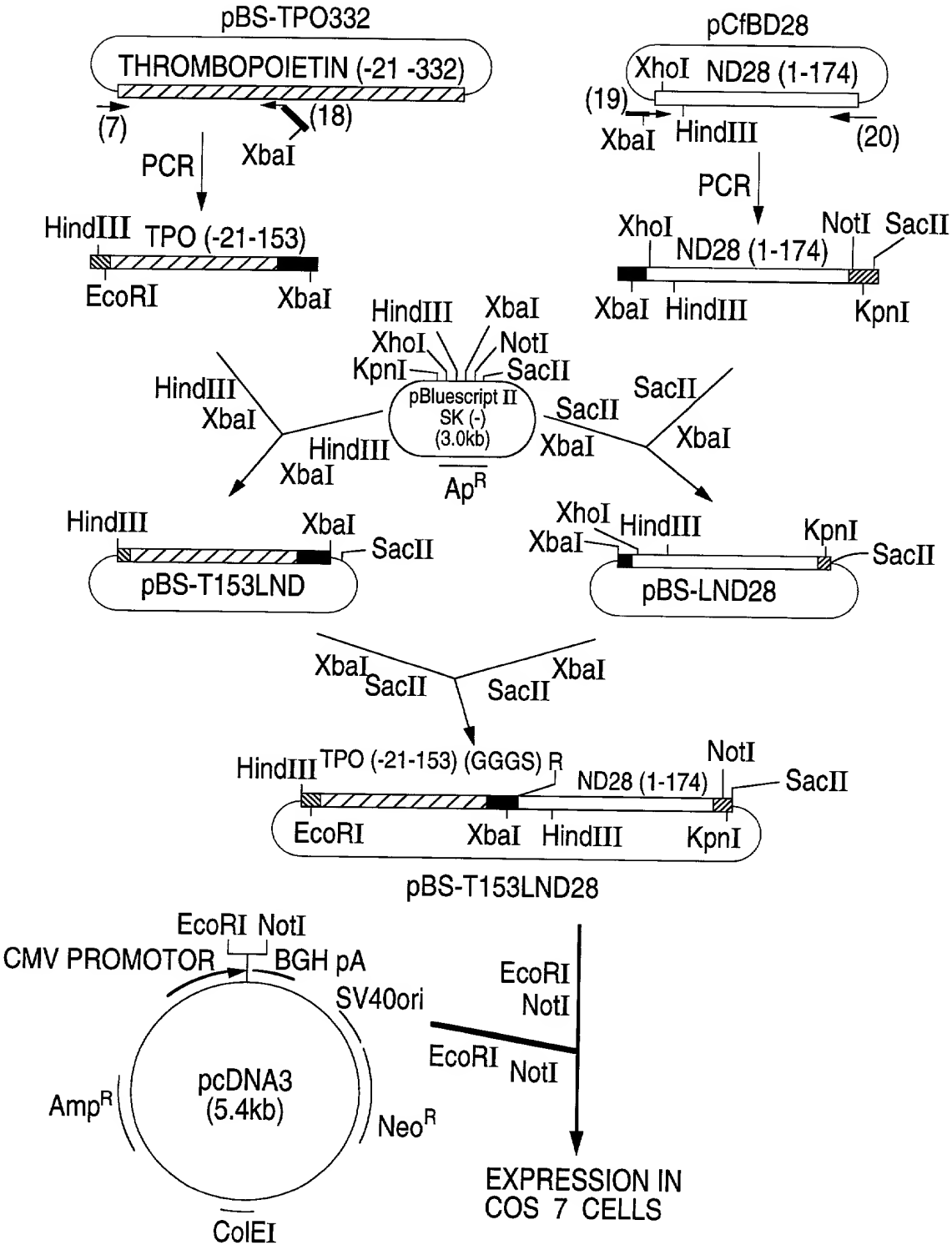

pBS-TPO332
THROMBOPOIETIN (-21 -332)
(7)
(18)
XbaI
PCR
HindIII TPO (-21-153)
EcoRI
XbaI
pCfBD28
XhoI ND28 (1-174)
(19)
XbaI HindIII
(20)
PCR
XhoI
NotI SacII
ND28 (1-174)
XbaI HindIII
KpnI
HindIII XbaI
XhoI
NotI
KpnI
SacII
HindIII
XbaI
pBluescript II
SK (-)
(3.0kb)
SacII
XbaI
HindIII
XbaI
SacII
XbaI
ApR
HindIII
XbaI
SacII
pBS-T153LND
XhoI HindIII
XbaI
KpnI
SacII
pBS-LND28
XbaI SacII
SacII XbaI
HindIII TPO (-21-153) (GGGS) R
ND28 (1-174)
NotI
SacII
EcoRI
XbaI HindIII
KpnI
pBS-T153LND28
EcoRI NotI
CMV PROMOTOR
BGH pA
SV40ori
pcDNA3
(5.4kb)
AmpR
NeoR
ColEI
EcoRI
NotI
EcoRI NotI
EXPRESSION IN
COS 7 CELLS

FIG. 2

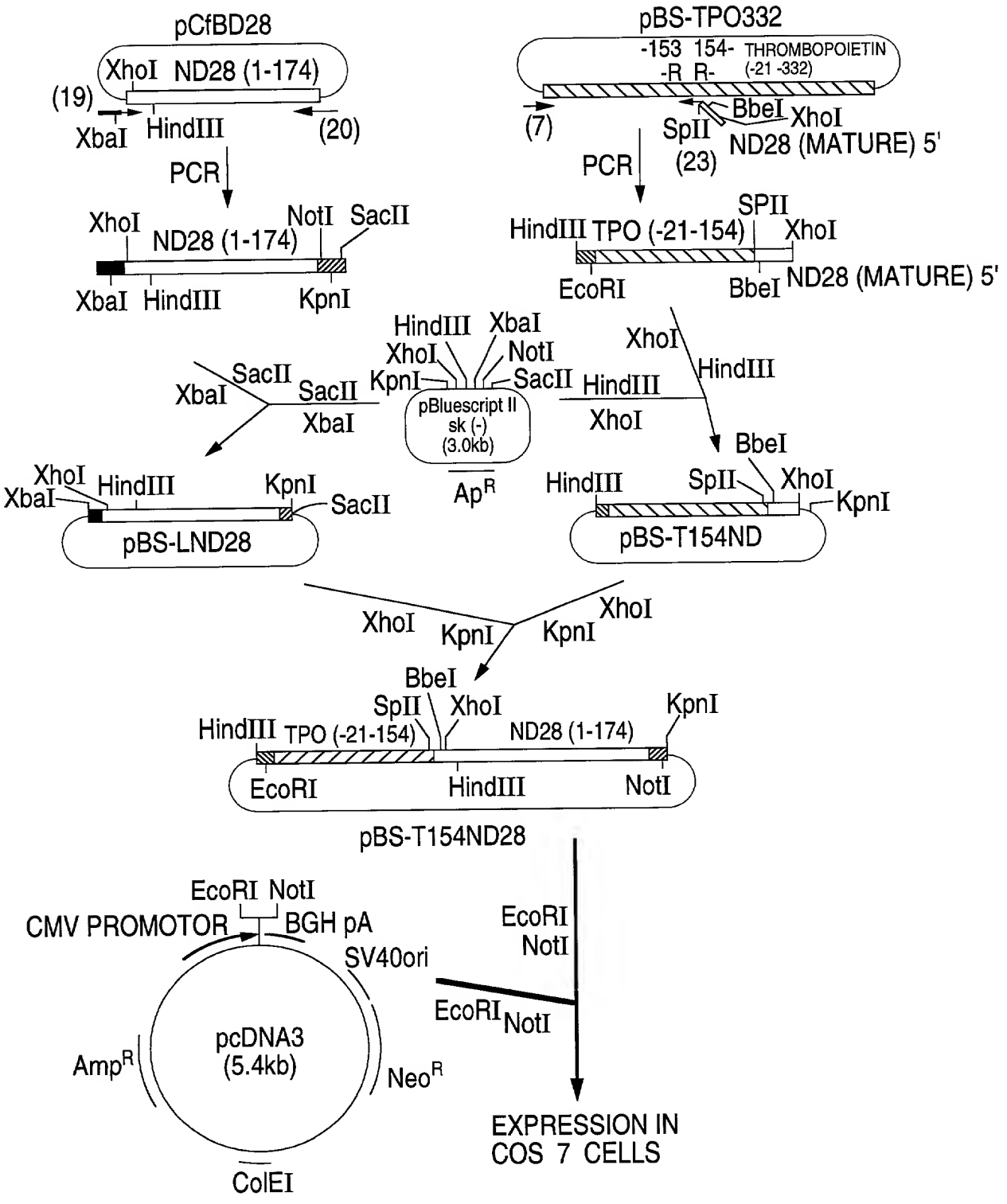
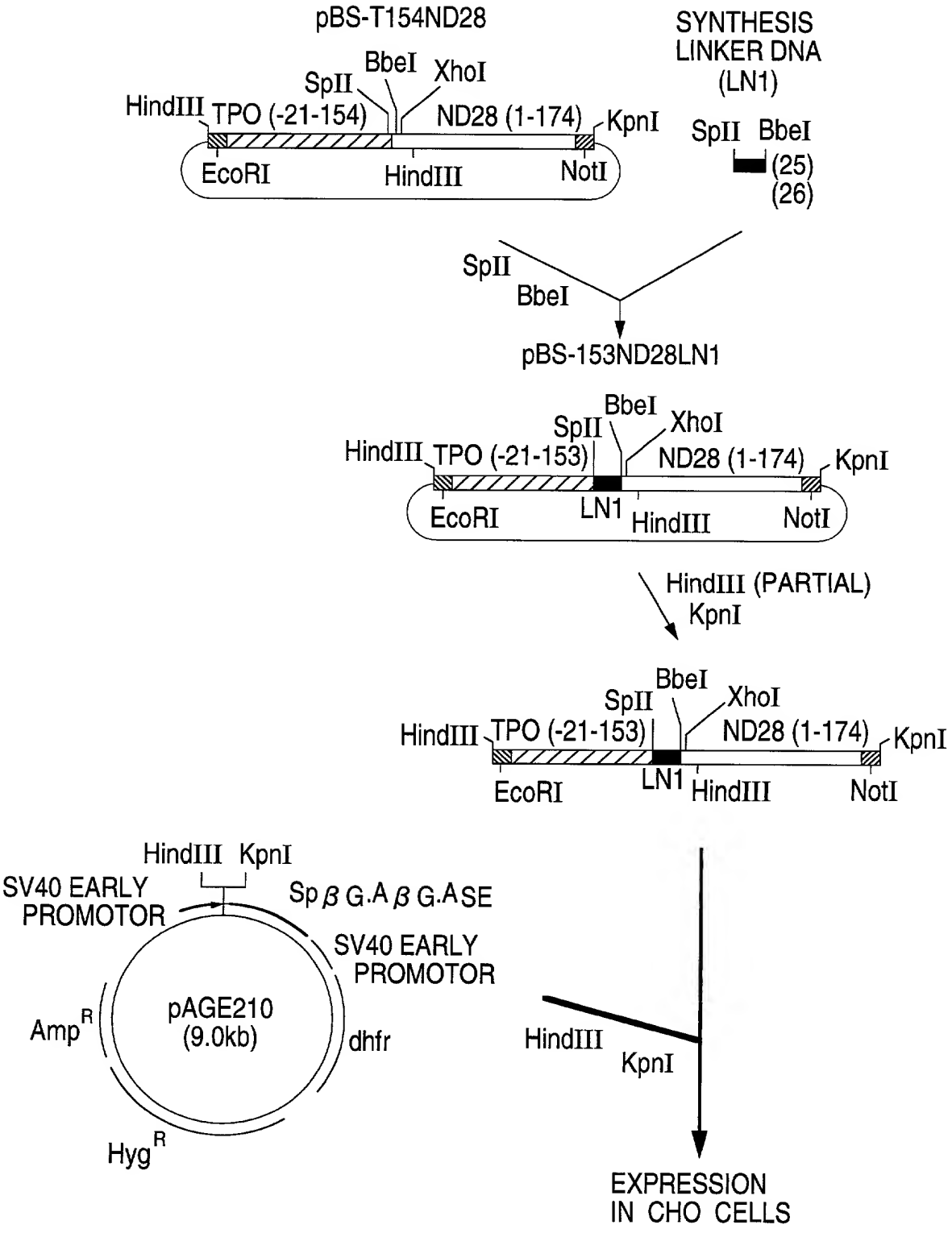

pCfBD28
XhoI ND28 (1-174)
(19)
XbaI HindIII
(20)
PCR
XhoI ND28 (1-174) NotI SacII
XbaI HindIII KpnI
pBS-TPO332
-153 154- THROMBOPOIETIN
-R R- (-21 -332)
(7)
SpII BbeI XhoI
ND28 (MATURE) 5'
PCR
(23)
SPII
HindIII TPO (-21-154) XhoI
EcoRI BbeI ND28 (MATURE) 5'
HindIII XbaI
XhoI NotI
KpnI SacII
SacII
XbaI
SacII
XbaI
pBluescript II
sk (-)
(3.0kb)
Ap^R
XhoI
HindIII
HindIII
XhoI
BbeI
XhoI HindIII KpnI
XbaI SacII
pBS-LND28
HindIII SpII XhoI KpnI
pBS-T154ND
XhoI KpnI KpnI XhoI
BbeI
SpII XhoI KpnI
HindIII TPO (-21-154) ND28 (1-174)
EcoRI HindIII NotI
pBS-T154ND28
EcoRI NotI
CMV PROMOTOR BGH pA
SV40ori
EcoRI NotI
pcDNA3
(5.4kb)
Amp^R
Neo^R
ColEI
EXPRESSION IN
COS 7 CELLS

FIG. 3


pBS-T154ND28
SpII
BbeI
XhoI
HindIII
TPO (-21-154)
ND28 (1-174)
KpnI
EcoRI
HindIII
NotI
SYNTHESIS
LINKER DNA
(LN1)
SpII
BbeI
(25)
(26)
SpII
BbeI
pBS-153ND28LN1
BbeI
SpII
XhoI
HindIII
TPO (-21-153)
ND28 (1-174)
KpnI
EcoRI
LN1
HindIII
NotI
HindIII (PARTIAL)
KpnI
BbeI
SpII
XhoI
HindIII
TPO (-21-153)
ND28 (1-174)
KpnI
EcoRI
LN1
HindIII
NotI
HindIII
KpnI
SV40 EARLY
PROMOTOR
SpβG.AβG.ASE
SV40 EARLY
PROMOTOR
pAGE210
(9.0kb)
Amp^R
dhfr
Hyg^R
HindIII
KpnI
EXPRESSION
IN CHO CELLS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

YOKOI et al — Atty. Ref.: 249-118

Serial No. to be assigned — Group: 1646

Filed: October 6, 2000 — Examiner: Mertz

For: HG-CSF FUSION POLYPEPTIDE HAVING C-MPL ACTIVITY, DNA CODING FOR SAME AND METHODS OF TREATING ANEMIA USING SAME(AS AMENDED)

* * * * * * * * * * *

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF DEPOSITED MATERIALS

As an authorized representative and on the instructions of Kyowa Hakko Kogyo Co., Ltd., a Japanese corporation of 6-1, Ohtemachi 1-chome, Chiyoda-ku, Tokyo, Japan, the assignee of the U.S. Patent application identified above, I hereby declare that:

- Kyowa Hakko Kogyo Co., Ltd. is the depositor and owner of the following deposits of biological materials identified and referred to in the specification of this application and on the attached deposit receipts, international form, under the terms of the Budapest Treaty.

Accession No. FERM-	Depositor's Reference	Date Deposited
FERM BP-5001	Escherichia coli TLN-1	February 16, 1995

- The deposits of biological material identified above were made at the National Institute of Bioscience and Human Technology; Agency of Industrial Science and Technology, Ibaraki, Japan, and have been deposited and accepted under the terms of the Budapest Treaty on the International Recognition of the Deposit of

Microorganisms for the Purposes of Patent Procedure and therefore the filing of a viability statement is unnecessary [37 C.F.R. 1.807(b)]

- The deposits will be maintained for a period of 30 years from the date of deposit or for the enforceable life of any patent issuing from this application or for a period of 5 years after the date of the most recent request for the furnishing of a sample of the deposited material, whichever is longest.
- The deposits will be replaced should they become contaminated or no longer viable.
- Subject to 37 C.F.R. § 1.808(b), all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent.
- Access to the deposited material is permitted during the pendency of the above-identified patent application to one determined by the Commissioner of Patents and Trademarks to be so entitled under 37 C.F.R. § 1.14 and 35 U.S.C. § 122.

I further declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____

Arthur R. Crawford
Reg. No. 25,327

Date: 10/6/00

Nixon & Vanderhye P.C. (6/92)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled NOVEL POLYPEPTIDES,

the specification of which (check applicable box(es)):

[X] is attached hereto.

[ ] was filed on _____ as U.S. Application Serial No. _____

[X] was filed as PCT international application No. PCT/JP96/01157 on April 26, 1996

and (if applicable to U.S. or PCT application) was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 C.F.R. 1.56(a). I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Prior Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
P. Hei. 7-102625	Japan	26/April/1995

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56(a) which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s):

Application Serial No.	Day/Month/Year Filed	Status: patented, pending, abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Road, 8th Floor, Arlington, Virginia 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Paul J. Henon, 33626; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Thomas E. Byrne, 32205.

1) Inventor's Signature Haruhiko Yokoi Date December 16, 1996

Inventor's Name (typed) Haruhiko (First) Yokoi (Middle Initial) Japan (Family Name / Citizenship)

Residence (City) Ibaraki (State/Foreign Country) Japan

Post Office Address 1-23-5, Sengen, Tsukuba-shi, Ibaraki, Japan Zip Code 305

2) Inventor's Signature Yukimasa Shiotsu Date December 16, 1996

Inventor's Name (typed) Yukimasa (First) Shiotsu (Middle Initial) Japan (Family Name / Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 1-17-20, Kinuta, Setagaya-ku, Tokyo, Japan Zip Code 157

Inventor's Signature Noboru Konishi Date December 16, 1996

Inventor's Name (typed) Noboru (First) Konishi (Middle Initial) Japan (Family Name / Citizenship)

Residence (City) Yamaguchi (State/Foreign Country) Japan

Post Office Address 535-12, Shinden, Hohu-shi, Yamaguchi, Japan Zip Code 747

FOR ADDITIONAL INVENTORS, check box [X] and attach sheet with same information and signature and date for each.

4) Inventor's Signature Hideharu Anazawa Date December 16, 1996

Inventor's Name (typed) Hideharu (First) Anazawa (Family Name) Japan (Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 4-19-18, Minami-ohizumi, Nerima-ku, Tokyo, Japan Zip Code 178

5) Inventor's Signature Tatsuya Tamaoki Date December 16, 1996

Inventor's Name (typed) Tatsuya (First) Tamaoki (Family Name) Japan (Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 2662-13, Honmachida, Machida-shi, Tokyo, Japan Zip Code 194

6) Inventor's Signature Motoo Yamasaki Date December 16, 1996

Inventor's Name (typed) Motoo (First) Yamasaki (Family Name) Japan (Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 3-9-13, Naka-machi, Machida-shi, Tokyo, Japan Zip Code 194

7) Inventor's Signature Yoko Kato Date December 16, 1996

Inventor's Name (typed) Yoko (First) Kato (Family Name) Japan (Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 1392-5, Honmachida, Machida-shi, Tokyo, Japan Zip Code 194

8) Inventor's Signature Kazuhisa Uchida Date December 16, 1996

Inventor's Name (typed) Kazuhisa (First) Uchida (Family Name) Japan (Citizenship)

Residence (City) Tokyo (State/Foreign Country) Japan

Post Office Address 2-12-3, Naruse, Machida-shi, Tokyo, Japan Zip Code 194

9) Inventor's Signature Kinya Yamashita Date December 16, 1996

Inventor's Name (typed) Kinya (First) Yamashita (Family Name) Japan (Citizenship)

Residence (City) Shizuoka (State/Foreign Country) Japan

Post Office Address 25-2, Sasahara-shinden, Mishima-shi, Shizuoka, Japan Zip Code 411

10) Inventor's Signature _____ Date _____

Inventor's Name (typed) _____ (First) _____ (Middle Initial) _____ (Family Name) _____ (Citizenship)

Residence (City) _____ (State/Foreign Country) _____

Post Office Address _____ Zip Code _____

11) Inventor's Signature _____ Date _____

Inventor's Name (typed) _____ (First) _____ (Middle Initial) _____ (Family Name) _____ (Citizenship)

Residence (City) _____ (State/Foreign Country) _____

Post Office Address _____ Zip Code _____

12) Inventor's Signature _____ Date _____

Inventor's Name (typed) _____ (First) _____ (Middle Initial) _____ (Family Name) _____ (Citizenship)

Residence (City) _____ (State/Foreign Country) _____

Post Office Address _____ Zip Code _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

YOKOI et al — Atty. Ref.: 249-118

Serial No. to be assigned — Group: 1646

Filed: October 6, 2000 — Examiner: Mertz

For: HG-CSF FUSION POLYPEPTIDE HAVING C-MPL ACTIVITY, DNA CODING FOR SAME AND METHODS OF TREATING ANEMIA USING SAME(AS AMENDED)

* * * * * * * * * * *

October 6, 2000

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

LETTER RE SEQUENCE LISTING

Pursuant to Rule 821(e) the applicants note the computer readable copy of the Sequence Listing of the present application is identical to the computer readable form of the Sequence Listing in the parent application Serial No. 08/765,337. The applicants request that the computer readable copy of the Sequence Listing from application Serial No. 08/765,337 be used in the present application.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

Arthur R. Crawford
Reg. No. 25,327

ARC:pfc
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: 249-89

In re patent application of

YOKOI, Haruhiko *et al*

Serial No. 08/765,337

Filed: December 23, 1996

For: NOVEL POLYPEPTIDES

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231
Box SEQUENCE

Sir:

In connection with a Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter;

2. the content of the attached paper copy and the attached computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same; and

3. all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

Oct. 1, 1997
Date

James A. Coburn

HARBOR CONSULTING
Intellectual Property Services
1500A Lafayette Road
Suite 262
Portsmouth, N.H.
800-318-3021

APPENDIX

- Original SEQ ID NOS 1-3 remain the same;
- Original SEQ ID NOS 4-6 are now SEQ ID NOS 4-5, 6-7 & 8-9, respectively;
- Original SEQ ID NO: 7 is now SEQ ID NO:10;
- Original SEQ ID NO: 8 is now SEQ ID NO:11;
- Original SEQ ID NO: 9 is now SEQ ID NO:12;
- Original SEQ ID NO: 10 is now SEQ ID NO:13;
  ***Please note that the part or all of the CDS regions for original SEQ ID NOS 8-10 were either incorrect or contained internal stop codons; since the amino acids were not explicitly listed in application, these coding regions were left out of the corrected Sequence Listing.**

- Original SEQ ID NO: 11 is now SEQ ID NOS 14-15;
- Original SEQ ID NO: 12 is now SEQ ID NOS 16-17;
- Original SEQ ID NO: 13 is now SEQ ID NOS 18-19;
- Original SEQ ID NO: 14 is now SEQ ID NOS 20-21;
- Original SEQ ID NOS 15-17 are now SEQ ID NOS 22-24;
- Original SEQ ID NOS 18-19 are now SEQ ID NOS 25-26 & 27-28, respectively;
- Original SEQ ID NO: 20 is now SEQ ID NO:29;
  ***See above note re: SEQ ID NOS 8-10.**

- Original SEQ ID NOS 21-22 are now SEQ ID NOS 30-31 & 32-33, respectively;
- Original SEQ ID NO: 23 is now SEQ ID NO:34;
  ***See above note re: SEQ ID NOS 8-10.**

- Original SEQ ID NO: 24 is now SEQ ID NO:35;
- Original SEQ ID NO: 25 is now SEQ ID NOS 36-37;
  ***See above note re: SEQ ID NOS 8-10.**

- Original SEQ ID NO: 26 is now SEQ ID NO:38;
  ***See above note re: SEQ ID NOS 8-10.**

- Table 1 encompasses SEQ ID NO:39;
- Table 3 encompasses SEQ ID NO:40;
- Table 4 encompasses SEQ ID NOS 24, 35 & 41-45, respectively.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Yokoi, Haruhiko
Shiotsu, Yukimasa
Konishi, Noboru

(ii) TITLE OF INVENTION: NOVEL POLYPEPTIDES

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: NIXON & VANDERHYE P.C.
(B) STREET: 1100 North Glebe Rd. 8th floor
(C) CITY: Arlington
(D) STATE: VA
(E) COUNTRY: USA
(F) ZIP: 22201-4741

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/765,337
(B) FILING DATE: 23-DEC-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/JP96/01157
(B) FILING DATE: 26-APR-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: JP P.HEI.7-102625
(B) FILING DATE: 26-APR-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Crawford, Arthur R.
(B) REGISTRATION NUMBER: 25,327
(C) REFERENCE/DOCKET NUMBER: 249-89

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 703-816-4000
(B) TELEFAX: 703-816-4100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 328 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
1               5                  10                  15

Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
            20                  25                  30

His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
        35                  40                  45

Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
    50                  55                  60

Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65                  70                  75                  80

Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
                85                  90                  95

Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
            100                 105                 110

Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
        115                 120                 125

Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
    130                 135                 140

Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala Pro Thr Tyr Arg Ala
145                 150                 155                 160

Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg
                165                 170                 175

Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr
            180                 185                 190

Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu
        195                 200                 205

Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln
    210                 215                 220

Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln
225                 230                 235                 240

Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr
                245                 250                 255

Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp
            260                 265                 270

Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln
        275                 280                 285

Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
    290                 295                 300
```

Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
305 310 315 320

Val Leu Arg His Leu Ala Gln Pro
325

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 340 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
1 5 10 15

Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
20 25 30

His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
35 40 45

Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
50 55 60

Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65 70 75 80

Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
85 90 95

Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
100 105 110

Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
115 120 125

Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
130 135 140

Val Gly Gly Ser Thr Leu Cys Val Arg Gly Gly Gly Ser Gly Gly Gly
145 150 155 160

Ser Gly Gly Gly Ser Arg Ala Pro Thr Tyr Arg Ala Ser Ser Leu Pro
165 170 175

Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg Lys Ile Gln Gly
180 185 190

Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys
195 200 205

His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp
210 215 220

Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys
225 230 235 240

Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln
245 250 255

Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu
260 265 270

Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu
275 280 285

Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro
290 295 300

Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala
305 310 315 320

Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His
325 330 335

Leu Ala Gln Pro
340

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 344 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
1 5 10 15

Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
20 25 30

His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
35 40 45

Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
50 55 60

Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65 70 75 80

Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
85 90 95

Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
100 105 110

```
Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
        115                 120                 125

Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
    130                 135                 140

Val Gly Gly Ser Thr Leu Cys Val Arg Ser Gly Gly Gly Ser Gly Gly
145                 150                 155                 160

Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg Ala Pro Thr Tyr Arg Ala
                165                 170                 175

Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val Arg
            180                 185                 190

Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala Thr
        195                 200                 205

Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser Leu
    210                 215                 220

Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln
225                 230                 235                 240

Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln
                245                 250                 255

Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr
            260                 265                 270

Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp
        275                 280                 285

Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln
    290                 295                 300

Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
305                 310                 315                 320

Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg
                325                 330                 335

Val Leu Arg His Leu Ala Gln Pro
            340
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1047 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..63

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 64..1047

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1047

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA        48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC        96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                   1                   5                  10

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC       144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT       192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG       240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG       288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75

GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG       336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90

CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC       384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                 100                 105

CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT       432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
        110                 115                 120

CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG       480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135

CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTA CGG CGG GCG       528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala
140                 145                 150                 155

CCA ACA TAT CGC GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC       576
Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
                160                 165                 170
```

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TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG      624
Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
            175                 180                 185

AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG      672
Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
        190                 195                 200

CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC      720
Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
    205                 210                 215

AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC      768
Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
220                 225                 230                 235

CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC      816
Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
                240                 245                 250

GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT      864
Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
            255                 260                 265

GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC      912
Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
        270                 275                 280

CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG      960
Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
    285                 290                 295

CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG     1008
Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
300                 305                 310                 315

GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC                 1047
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
                320                 325
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 349 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                  1                   5                  10

Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25
```

Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
30 35 40

Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
45 50 55

Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
60 65 70 75

Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
80 85 90

Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
95 100 105

Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
110 115 120

Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
125 130 135

Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala
140 145 150 155

Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
160 165 170

Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
175 180 185

Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
190 195 200

Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
205 210 215

Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
220 225 230 235

Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
240 245 250

Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
255 260 265

Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
270 275 280

Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
285 290 295

Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
300 305 310 315

Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
320 325

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1083 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..63

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 64..1083

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1083

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA        48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC        96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                   1               5                  10

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC       144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT       192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG       240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG       288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75

GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG       336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90

CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC       384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                  100                 105

CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT       432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
         110                 115                 120
```

```
CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG        480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135

CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTC AGG GGT GGC        528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Gly Gly
140                 145                 150                 155

GGT TCT GGA GGT GGT TCC GGA GGG GGT TCT AGA GCA CCA ACA TAT CGC        576
Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg Ala Pro Thr Tyr Arg
                160                 165                 170

GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC TTA GAG CAA GTG        624
Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val
            175                 180                 185

AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG AAG CTG TGT GCC        672
Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala
        190                 195                 200

ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG CTC GGA CAC TCT        720
Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser
    205                 210                 215

CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC AGC CAG GCC CTG        768
Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu
220                 225                 230                 235

CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC        816
Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr
                240                 245                 250

CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC GAG TTG GGT CCC        864
Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro
            255                 260                 265

ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC        912
Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile
        270                 275                 280

TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC        960
Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr
    285                 290                 295

CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA       1008
Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly
300                 305                 310                 315

GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC       1056
Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr
                320                 325                 330

CGC GTT CTA CGC CAC CTT GCC CAG CCC                                   1083
Arg Val Leu Arg His Leu Ala Gln Pro
            335                 340
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 361 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                   1                   5                  10

Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75

Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90

Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                  100                 105

Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
         110                 115                 120

Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
     125                 130                 135

Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Gly Gly
140                 145                 150                 155

Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg Ala Pro Thr Tyr Arg
                160                 165                 170

Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser Leu Glu Gln Val
            175                 180                 185

Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys Leu Cys Ala
        190                 195                 200

Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly His Ser
    205                 210                 215

Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu
220                 225                 230                 235

Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr
                240                 245                 250

Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro
            255                 260                 265
```

```
Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile
        270                 275                 280

Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr
    285                 290                 295

Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly
300                 305                 310                 315

Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr
                320                 325                 330

Arg Val Leu Arg His Leu Ala Gln Pro
            335                 340
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1095 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 64..1095

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1095

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
ATG GAG CTG ACT GAA TTG CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA        48
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

AGG CTA ACG CTG TCC AGC CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC        96
Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                  1                   5                  10

CTC AGT AAA CTG CTT CGT GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC       144
Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

CAG TGC CCA GAG GTT CAC CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT       192
Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

GTG GAC TTT AGC TTG GGA GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG       240
Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

GCA CAG GAC ATT CTG GGA GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG       288
Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75
```

```
GCA GCA CGG GGA CAA CTG GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG        336
Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                80                  85                  90

CAG CTT TCT GGA CAG GTC CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC        384
Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
            95                  100                 105

CTT GGA ACC CAG CTT CCT CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT        432
Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
        110                 115                 120

CCC AAT GCC ATC TTC CTG AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG        480
Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135

CGT TTC CTG ATG CTT GTA GGA GGG TCC ACC CTC TGC GTA CGG TCC GGA        528
Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Ser Gly
140                 145                 150                 155

GGT GGC TCT GGC GGT GGT TCT GGT GGC GGC TCC GGA GGC GGT CGT GCG        576
Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg Ala
                160                 165                 170

CCA ACA TAT CGC GCC TCG AGT CTA CCA CAG AGC TTC CTT TTA AAA AGC        624
Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
            175                 180                 185

TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC CAG GAG        672
Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
        190                 195                 200

AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG CTG GTG CTG        720
Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
    205                 210                 215

CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG AGC AGC TGC CCC        768
Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
220                 225                 230                 235

AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC        816
Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
                240                 245                 250

CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC        864
Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
            255                 260                 265

GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC TTT        912
Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
        270                 275                 280

GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC        960
Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
    285                 290                 295

CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG       1008
Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
300                 305                 310                 315
```

```
CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG      1056
Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
                320                 325                 330

GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC                 1095
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
            335                 340
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                  1                   5                  10

Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75

Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90

Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                  100                 105

Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
        110                 115                 120

Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135

Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Ser Gly
140                 145                 150                 155

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Arg Ala
                160                 165                 170

Pro Thr Tyr Arg Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Ser
            175                 180                 185

Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu
        190                 195                 200
```

Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu
205 210 215

Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro
220 225 230 235

Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly
240 245 250

Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro
255 260 265

Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe
270 275 280

Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala
285 290 295

Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln
300 305 310 315

Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
320 325 330

Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
335 340

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 27..44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTCTCCAAGC TTGAATTCCG GCCAGAATGG AGCTGACTGA ATTG 44

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAGAGGTAC CGCGGCCGCT TACCCTTCCT GAGACAGATT CTGGGAG 47

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGAACCTCTG GGCACTGGCT CAGT 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTGCCTGCT GTGGACTTTA GCTT 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
TGT TGG AAG CTC AGG AAG ATG GCA                                          24
Cys Trp Lys Leu Arg Lys Met Ala
  1               5
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Cys Trp Lys Leu Arg Lys Met Ala
  1               5
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
CCT GAT GCT TGT AGG AGG GTC CAC                                          24
Pro Asp Ala Cys Arg Arg Val His
  1               5
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Pro Asp Ala Cys Arg Arg Val His
  1               5
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
TCA AGA GTT CGT GTA TCC TGT TCA                                    24
Ser Arg Val Arg Val Ser Cys Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Ser Arg Val Arg Val Ser Cys Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
GAA TGG AAC TCG TGG ACT CTT TCC                                    24
Glu Trp Asn Ser Trp Thr Leu Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Trp Asn Ser Trp Thr Leu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CAGGAAACAG CTATGAC 17

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Arg
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..3, 43..66)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
TGC TCTAGAACCG CCTCCGGAAC CACCTCCAGA ACCGCCACC CCT GAC GCA GAG          54
Cys                                              Pro Asp Ala Glu
  1                                                              5

GGT GGA CCC TCC                                                        66
Gly Gly Pro Ser
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Cys Pro Asp Ala Glu Gly Gly Pro Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 22..45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGTTCCGGAG GCGGTTCTAG A GCA CCA ACA TAT CGC GCC TCG AGT 45
Ala Pro Thr Tyr Arg Ala Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Pro Thr Tyr Arg Ala Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATTCCGCGG GGTACCGCGG CCGCTCAGGG CTGGGCAAGG TGGCGTAG 48

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGC TGC TTG AGC CAA CTC CAT AGC 24
Gly Cys Leu Ser Gln Leu His Ser
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Cys Leu Ser Gln Leu His Ser
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAC CCA ACT CGG GGG AGA TCC CTT 24
Asp Pro Thr Arg Gly Arg Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Asp Pro Thr Arg Gly Arg Ser Leu
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(25, "")

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(33..34, "")

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TAGACTCGAG GCGCGATATG TTGGCGCCCG CCGTACGCAG AGGGTGGACC CTCCTAC 57

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Arg

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..6

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 7..57

(D) OTHER INFORMATION: /product= "linker peptide"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /product= "SplI"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 7..12
(D) OTHER INFORMATION: /product= "MroI"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 43..48
(D) OTHER INFORMATION: /product= "MroI"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 58..61
(D) OTHER INFORMATION: /product= "BbeI"

(ix) FEATURE:
(A) NAME/KEY: mutation
(B) LOCATION: replace(4..5, "")

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTA CGG TCCGGAGGTG GCTCTGGCGG TGGTTCTGGT GGCGGCTCCG GAGGCGGTCG 56
Val Arg
1

TGCGC 61

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Val Arg
1

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..51
(D) OTHER INFORMATION: /product= "linker peptide"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 53
(D) OTHER INFORMATION: /product= "SplI"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 10..15
(D) OTHER INFORMATION: /product= "MroI"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 46..51
(D) OTHER INFORMATION: /product= "MroI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ACGACCGCCT CCGGAGCCGC CACCAGAACC ACCGCCAGAG CCACCTCCGG ACC 53

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Xaa Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu
1 5 10 15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu
20 25 30

Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Gly Leu
35 40 45

Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser
50 55 60

Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His
65 70 75 80

Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
85 90 95

Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala
100 105 110

Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala
115 120 125

Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala
130 135 140

Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser
145 150 155 160

Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170 175

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 332 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu
1 5 10 15

Arg Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val
20 25 30

His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu
35 40 45

Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu
50 55 60

Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln
65 70 75 80

Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln
85 90 95

Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu
100 105 110

Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe
115 120 125

Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu
130 135 140

Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala Pro Pro Thr Thr Ala
145 150 155 160

Val Pro Ser Arg Thr Ser Leu Val Leu Thr Leu Asn Glu Leu Pro Asn
165 170 175

```
Arg Thr Ser Gly Leu Leu Glu Thr Asn Phe Thr Ala Ser Ala Arg Thr
            180                 185                 190

Thr Gly Ser Gly Leu Leu Lys Trp Gln Gln Gly Phe Arg Ala Lys Ile
        195                 200                 205

Pro Gly Leu Leu Asn Gln Thr Ser Arg Ser Leu Asp Gln Ile Pro Gly
    210                 215                 220

Tyr Leu Asn Arg Ile His Glu Leu Leu Asn Gly Thr Arg Gly Leu Phe
225                 230                 235                 240

Pro Gly Pro Ser Arg Arg Thr Leu Gly Ala Pro Asp Ile Ser Ser Gly
                245                 250                 255

Thr Ser Asp Thr Gly Ser Leu Pro Pro Asn Leu Gln Pro Gly Tyr Ser
            260                 265                 270

Pro Ser Pro Thr His Pro Pro Thr Gly Gln Tyr Thr Leu Phe Pro Leu
        275                 280                 285

Pro Pro Thr Leu Pro Thr Pro Val Val Gln Leu His Pro Leu Leu Pro
    290                 295                 300

Asp Pro Ser Ala Pro Thr Pro Thr Pro Thr Ser Pro Leu Leu Asn Thr
305                 310                 315                 320

Ser Tyr Thr His Ser Gln Asn Leu Ser Gln Glu Gly
                325                 330
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Ser Gly Gly Gly Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ser Gly Gly Gly
1

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Gly Gly Ser Gly Gly Gly Ser
1 5